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STUDIES ON THE NATURE OF RUST RESISTANCE IN WHEAT

VII. CHEMICAL ANALYSES OF HYBRID LINES OF WHEAT DIFFERING IN THEIR RUST REACTIONS[1]

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Abstract

Four lots of wheat, representing the four possible combinations of seedling and mature-plant reaction to rust, were grown in the field, and leaves were collected at the seedling stage and after heading. The material was dried, ground and analyzed for:— total ash; total nitrogen; fat; cold- and hot-water-soluble organic matter, ash, reducing compounds, reducing sugars and invert sugar; alcohol-soluble matter; reducing compounds (from hemicelluloses) and nitrogen liberated by hydrochloric acid; reducing compounds (from cellulose) and nitrogen liberated by sulphuric acid; and for ash, protein and lignin (by difference) in the remaining residue. The material was also subjected to quantitative extraction with the following solvents in series:— ligroin, ether, chloroform, ethyl acetate, acetone, and ethyl alcohol.

Small but significant differences in constitution were found between the wheat classes. There was no evidence that these differences in constitution were related to rust reaction.

Introduction

Physiological susceptibility and resistance to stem rust in wheat appear to depend upon a balance or lack of balance between the physiological processes of the host and those of the parasite. The complicated relations which exist between the various wheat varieties and physiologic forms of the fungus preclude the possibility of any simple explanation of resistance and susceptibility. It seems probable that variations in physiology exist amongst both the wheat varieties and the rust forms, and that it is the combinations and permutations of these that give rise to the complicated relations which exist between host and parasite. If this be true, no solution of the problem of the fundamental nature of rust resistance can be expected until adequate studies have been made of the physiological processes of a wide range of susceptible and resistant wheat varieties and of a large number of physiologic forms of the fungus.

The chemist can contribute to these studies by analyzing the host tissues. It is true that such analyses must be carried out on dead material, but it must be borne in mind that the dead plant is a partial record of the metabolic activities of the protoplasm. A study of the compounds present in the dead tissue, which are end products of the biochemical reactions which have taken place, provides one method of comparing the physiology of different plants.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Parts I-VI in this series were published in this Journal, 1 : 13-35, 86-99, 1929; 4 : 134-147, 1931; 11 : 667-686, 1934.

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For many years, chemical investigations of the nature of rust resistance were unattractive since they were almost foredoomed to failure because of the nature of the material available for analysis. In order to obtain a series of wheat varieties differing widely in their reactions to stem rust, it was necessary to select representatives of several species of wheat. Resistant varieties were taken from the emmer group and susceptible varieties from the *vulgare* group. The interpretation of the results of analyses was thus complicated by the fact that such differences as might be found might easily represent morphological or physiological characters associated with the class of wheat rather than with rust reactions.

During the past few years plant breeders working on the development of new rust-resistant wheat varieties have produced material from which the chemist can select far more suitable series of wheats for his investigations. Such a series was obtained about three years ago from the Dominion Rust Research Laboratory, Winnipeg. It consists of twelve hybrid lines from a Marquis × H-44-24 cross which may be grouped into four classes representing the four possible combinations of seedling and mature-plant reaction to stem rust. These lines are homozygous for rust reaction but with respect to morphological characters represent random selections in the F_2 generation. In the F_3 generation they were found to be true breeding for rust resistance, and since then have been carried along in bulk without re-selection of any kind. They are thus pure lines for rust resistance only and for morphological characters consist of random mixtures of pure lines together with a number of heterozygous plants. The result is an equalization of differences between strains for all characters except rust resistance and other characters linked to it closely.

It is apparent that if all the emmer characters of the H-44-24 were carried by one chromosome and that if this chromosome had come over intact to the rust resistant lines, this material might be no better for comparative tests than different wheat varieties since there might still be incidental correlations between rust resistance and chemical compounds. The fact that the two types of rust resistance and several morphological characters have been shown to be inherited independently (1, 3) is evidence that the emmer characters did not come over in one chromosome and, consequently, the probability that a certain chemical compound or constitution characteristic of the emmer species is fortuitously linked with rust resistance in this material, is very much reduced.

When investigations reported in this paper were undertaken it was expected that time and staff would be available for extended analytical studies of these wheats. In these circumstances it appeared wise to prepare for more difficult examinations by first subjecting the wheats to a comparatively simple series of analyses designed to determine the main groups of plant compounds: fats, sugars, nitrogen compounds, hemicellulose, cellulose, lignin, etc. Although it seemed scarcely probable that differences related to rust reaction would be found between the wheats in these constituents, the work was undertaken in

the belief that it constituted the most logical approach to the problem under investigation.

Owing to the pressure of other work, these investigations now have to be dropped for some years at least. Meanwhile the results so far obtained are published in the hope that they may be of use to other investigators.

Materials

Production

A classification of the twelve lines of wheat described above, together with the letters by means of which each class will be identified throughout the rest of this paper, is presented in Table I. The seed for each class was made up by mixing equal weights of the seed of each of the three lines composing the class. The crop was grown on the experimental fields of the Department of Field Crops, University of Alberta, Edmonton, during 1933. The wheats were seeded in a block consisting of rod-row plots side by side. The block was divided into five sub-blocks, each containing four plots of three rows. Each sub-block contained one plot of each class of wheat, the classes being distributed at random within sub-blocks. All plots were seeded on May 2, 1933, at a rate of two bushels per acre. Three rows of Marquis wheat were seeded around the entire block.

TABLE I

CLASSIFICATION OF HYBRID LINES OF WHEAT SELECTED FROM A MARQUIS × H-44-24 CROSS

Winnipeg, 1929 Greenhouse No.	Rust reaction		Identification letters
	Seedling	Mature-plant	
113 125 135	Resistant	Resistant	RR
91 347 702	Resistant	Susceptible	RS
111 121 149	Susceptible	Resistant	SR
127 263 637	Susceptible	Susceptible	SS

Collection of seedling leaves

The first collection was made on May 27 when the plants were at the three-leaf stage. The leaves were cut off just above ground level, precautions being taken to see that no soil was included with the samples. A sample of 100 gm. green weight, was cut from each plot.

Collection of mature leaves

Mature leaves were collected on July 8 when the plants were well headed out. Samples were taken from plants which had not previously been cut. The leaves, with the exception of the terminal one (which does not exhibit the same degree of mature-plant resistance), were stripped off by hand. All dead and brown parts were discarded and a 100-gm. sample of green leaves was taken from each plot.

Methods

The analyses were carried out according to the system outlined by Waksman and Stevens (5), with the addition of a series of quantitative extractions with organic solvents. The analytical procedure is shown in the form of a flow sheet on page 5.

Dry matter

Since there were five plots of each class of wheat, determinations of air-dry matter were made in quintuplicate. Immediately after cutting, 100-gm. samples of the green leaves were placed in tared, loose, cheesecloth bags and dried first for 1.5 hr. at 110° C., then for three days at 65° C. After standing at room temperature for four days, the material reached an equilibrium with room conditions. It was then weighed and the percentage air-dry matter was calculated.

The material was then shipped to Ottawa where the five samples of each class of wheat were combined and ground in a Wiley mill to pass a 60-mesh sieve.

Oven-dry matter was determined in duplicate on the bulk samples by drying for 24 hr. *in vacuo* at 98° C.

Total ash

Determinations of total ash were carried out in duplicate by burning 1.5-gm. samples to constant weight in a muffle furnace at 600° C.

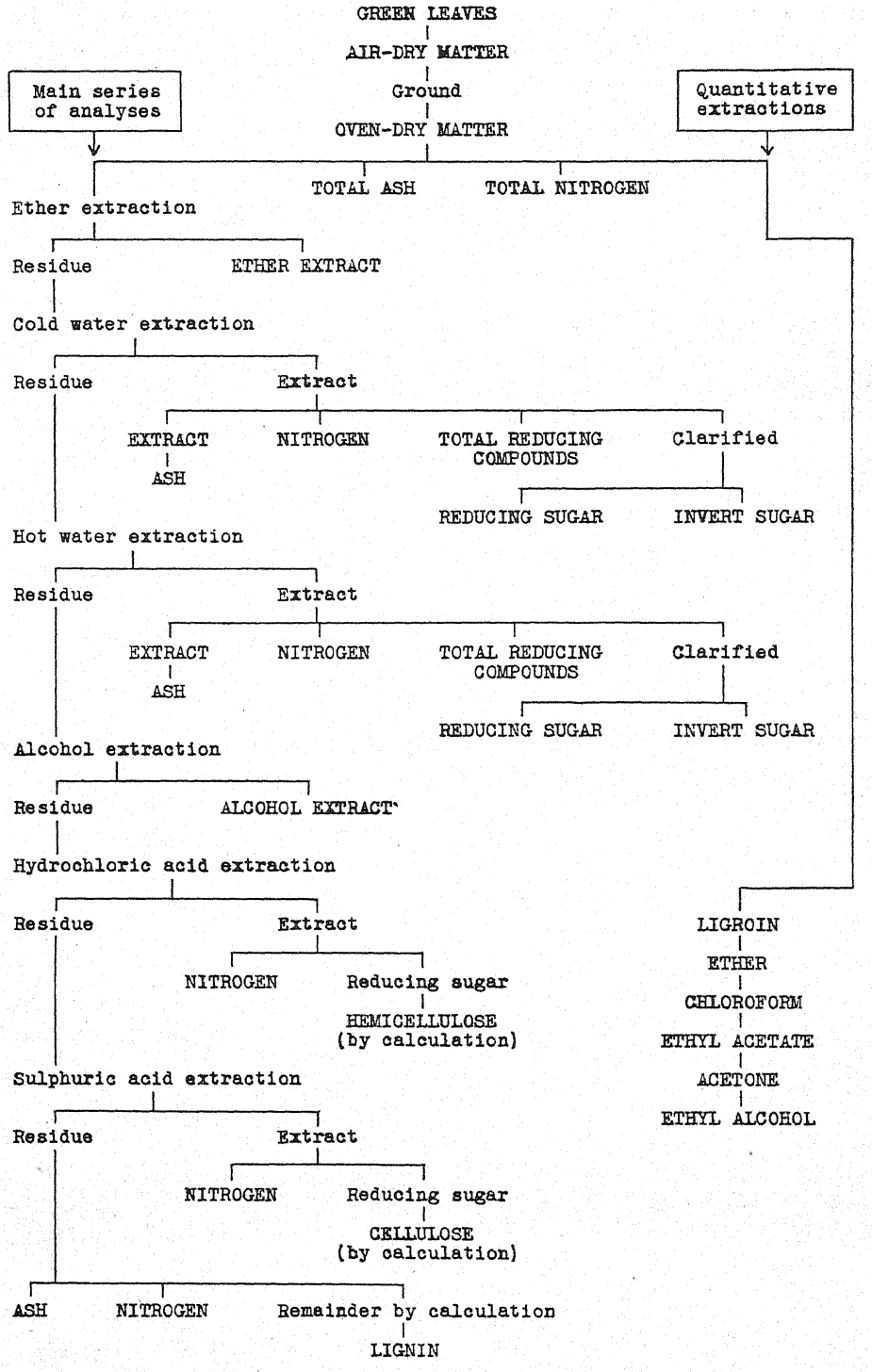
Total nitrogen

Determinations of total nitrogen were carried out on duplicate 2-gm. samples by the reduced iron modification of the Kjeldahl method (4).

Main series of analyses

The cold-water, hot-water, alcohol, and acid extractions outlined by Waksman and Stevens (5) are empirical and require the most painstaking technique if duplicate analyses are to check closely. Experiments showed that determinations made on different days were not directly comparable, owing, doubtless, to small and uncontrollable differences in conditions. In order to abolish this source of error, the eight samples, representing duplicates of each of the four wheat classes cut at the same stage of growth, were analyzed concurrently. In addition, the possibility of systematic errors, introduced by the order of precedence in handling, was minimized by randomizing the order in which the eight samples were dealt with throughout the various steps of the analytical procedure. Where heating was necessary, it was carried out in a mechanically stirred, electrically heated and controlled bath filled with either water or oil.

The cold- and hot-water extracts filtered slowly and fermented rapidly, thus increasing the difficulties of the determinations by setting time limits for the operation involved from the time the cold water was added to the sample until it was treated with alcohol. The speed of filtration was increased by extracting all samples in centrifuge bottles and by centrifuging and decanting


GREEN LEAVES
AIR-DRY MATTER
Ground
OVEN-DRY MATTER
Main series of analyses
Quantitative extractions
TOTAL ASH
TOTAL NITROGEN
Ether extraction
Residue
ETHER EXTRACT
Cold water extraction
Residue
Extract
EXTRACT
ASH
NITROGEN
TOTAL REDUCING COMPOUNDS
Clarified
REDUCING SUGAR
INVERT SUGAR
Hot water extraction
Residue
Extract
EXTRACT
ASH
NITROGEN
TOTAL REDUCING COMPOUNDS
Clarified
REDUCING SUGAR
INVERT SUGAR
Alcohol extraction
Residue
ALCOHOL EXTRACT
Hydrochloric acid extraction
Residue
Extract
NITROGEN
Reducing sugar
HEMICELLULOSE (by calculation)
Sulphuric acid extraction
Residue
Extract
NITROGEN
Reducing sugar
CELLULOSE (by calculation)
ASH
NITROGEN
Remainder by calculation
LIGNIN
LIGROIN
ETHER
CHLOROFORM
ETHYL ACETATE
ACETONE
ETHYL ALCOHOL

the extract on to the filter. Washing was accomplished by the same procedure, the remainder of the residue being transferred to the filter with the last washings. Fermentation was prevented for the required period by the use of chloroform water. The necessary technical accuracy and a standard programme for the execution of the various analyses were developed by means of preliminary experiments.

The initial weight of the samples taken for analysis was 5 gm. Ether extract was determined indirectly by the method described later. The cold-water- and hot-water-soluble matter were determined directly by evaporating aliquots of the extracts to dryness in Vitreosil dishes. Ash determinations were made on the residue in the same dish by the method previously described. Alcohol-soluble matter was determined by evaporating the whole of the extract to dryness in a glass beaker. The hydrochloric-acid- and sulphuric-acid-soluble matter were determined indirectly by weighing the sample before and after extraction. Nitrogen determinations were made on aliquots of the various extracts by the method previously described.

All sugar determinations were carried out on aliquots of the extracts by Hulme and Narain's (2) modification of the Hagedorn-Jensen-Hanes method. Clarification was effected with a minimum quantity of basic lead acetate solution (the quantity having been determined beforehand in a pilot experiment), and the solutions were de-leaded with sodium hydrogen phosphate. Total reducing compounds were determined by the same method without clarification. Inversion was carried out for 24 hr. at room temperature with hydrochloric acid (5 ml., sp. gr. 1.184, in 50 ml. of solution) and neutralization was effected by adding from a pipette 25 ml. of sodium hydroxide solution of the exact strength required to neutralize the acid solution.

The determinations of total reducing compounds in the cold- and hot-water extracts, and of the nitrogen in the acid extracts, were the only additions to the system of analyses outlined by Waksman and Stevens (5). In all other respects their directions were followed.

Quantitative extractions

The Soxhlet apparatus used for the quantitative extractions consisted of eight extractors, with interchangeable ground glass joints, arranged in a compact circle in a mechanically stirred, electrically heated and controlled water bath. The extractors were carefully selected for uniformity in size and rate of siphoning. Uniform boiling was promoted by placing a 1-cm. cube of pumice stone in the bottom of each flask. Extraction flasks, extraction units, condensers and pumice stone blocks were numbered and each set was kept together and was always placed in the same position in the bath. The stand for the extractors was made in such a way that all extractors were immersed to the same depth and could be lowered into the bath, or raised out of it, at the same time.

The determinations were carried out by the indirect method. The samples (initial weight 4 gm.) were dried, both before and after extraction, for 24 hr. *in vacuo* at 98° C. Extraction was continued for 48 hr. with each solvent.

After extraction with each solvent the replicate residues were mixed and re-sampled for the next extraction. This method was adopted to prevent the introduction of correlated errors.

The seedling and mature samples were analyzed in separate series, each of which consisted of either two or three sets of duplicate extractions of the four classes of wheat. Three sets of extractions were made with ligroin, ether and chloroform, and two with ethyl acetate, acetone and ethyl alcohol. The order in which the samples were weighed out for each set was randomized and the same order was maintained for all succeeding operations. Since the extractors were numbered and kept in order, the distribution of the samples in them was also randomized by this procedure.

Analytical Results

The analytical results are presented in Table II as the means of replicate determinations. The differences between means required for a 5% level of significance were calculated by statistical methods, and they are also reported in the table. Since these differences show the standard of accuracy attained in each determination, the publication of results of replicate determinations is unnecessary. In those cases in which the determination was not sufficiently precise to differentiate between any of the varieties the differences are enclosed in brackets.

With the exception of the quantitative extractions, all determinations were carried out in duplicate and concurrently on four classes of wheat collected at the same stage of growth. Four pairs of duplicate analyses were thus available in each case for statistical treatment. The standard deviation of the mean of duplicate determinations was calculated from these data and hence the necessary difference between means required for a 5% level of significance.

Quantitative extractions were carried out concurrently on duplicate samples of each of the four classes of wheat, collected at the same stage of growth. The extractions were then repeated either once or twice more. The data from either two or three sets of four duplicate analyses were thus available for statistical treatment. The data were subjected to an analysis of variance, the variance being divided into portions due to: (i) differences in the general level of results obtained in different sets of analyses, (ii) average differences, over all sets, between wheat classes, (iii) variations in differences between wheat classes from set to set, and (iv) differences between duplicate determinations within sets. The variance due to differences between sets of analyses as a whole was thus eliminated from the comparison of the wheat classes. The Z test was then applied to determine whether the variance due to wheat classes was significantly greater than those due to the interaction and the variance within sets. When the results of the Z test were positive the necessary difference between means, required for a 5% level of significance, was calculated in the usual manner.

TABLE II

ANALYSES OF HYBRID LINES OF WHEAT, DIFFERING IN THEIR RUST REACTIONS, AT TWO STAGES OF GROWTH

Determination	Wheat class, seedling				Necessary difference*	Wheat class, mature				Necessary difference*
	RR	RS	SR	SS		RR	RS	SR	SS	
Dry matter										
Air-dry matter as % of green wt.	18.40	18.77	18.48	18.53	(0.64)	24.81	24.41	23.94	23.61	0.96
Oven-dry matter as % of A.D. matter	92.24	92.40	92.28	91.49	0.08	94.17	93.84	93.79	93.87	0.12
Oven-dry matter as % of green wt.	19.95	20.31	20.03	20.25	—	26.35	25.80	25.52	25.15	—
Main series of analyses (Constituents as % of oven-dry matter)										
Total ash	14.13	13.58	14.12	13.65	0.09	8.18	8.22	8.32	8.27	0.05
Total nitrogen	6.19	6.15	6.13	6.23	0.03	2.25	2.35	2.30	2.24	0.02
Ether extract	5.09	5.24	5.23	5.40	0.09	2.28	2.21	2.26	2.13	0.03
Cold water extract	37.05	36.40	37.43	36.61	0.17	22.32	21.96	21.89	21.47	0.27
Ash	10.32	10.23	10.04	10.38	(0.76)	6.05	6.12	6.16	6.31	0.08
Nitrogen	1.51	1.40	1.48	1.48	0.03	0.90	0.93	0.94	0.91	0.03
Total reducing compounds	3.94	3.76	4.20	4.04	0.08	5.67	5.60	5.22	5.26	0.07
Reducing sugars	1.16	0.96	1.32	1.21	0.07	4.02	3.97	3.69	3.70	0.13
Invert sugar	6.63	7.07	7.01	6.83	0.15	3.31	2.75	3.09	2.91	0.11
Hot water extract	3.11	3.04	3.21	3.16	(0.33)	2.40	2.50	2.49	2.38	0.09
Ash	0.98	0.96	1.07	1.01	(0.12)	0.60	0.65	0.63	0.64	0.02
Nitrogen	0.16	0.15	0.15	0.16	(0.02)	0.07	0.08	0.07	0.07	0.01
Total reducing compounds	0.55	0.52	0.57	0.56	0.05	0.62	0.65	0.61	0.62	0.01
Reducing sugars	0.17	0.13	0.16†	0.17	—	0.37	0.37	0.36	0.36	(0.04)
Invert sugar	0.07	0.07	0.08†	0.08	—	0.08	0.07	0.09	0.08	(0.03)
Alcohol extract	3.37	3.57	3.36	3.41	0.11	2.31	2.25	2.39	2.21	0.05
Hydrochloric acid extract	34.29	34.49	33.66	34.13	0.25	33.28	33.28	33.04	33.37	(0.76)
Nitrogen	3.75	3.74	3.69	3.77	0.05	0.89	0.93	0.88	0.88	0.02
Hemicellulose	10.95	11.00	10.55	10.81	0.07	22.07	22.14	22.19	22.48	0.23
Sulphuric acid extract	12.21	12.19	12.07	12.36	(0.52)	28.78	28.51	29.41	29.47	0.62
Nitrogen	0.33	0.31	0.34	0.33	0.01	0.12	0.12	0.12	0.11	(0.02)
Cellulose	8.83	8.85	8.74	8.93	0.19	24.32	24.11	24.91	25.10	0.47
Residue	5.12	4.94	5.30	4.89	(0.49)	8.32	8.82	8.29	8.47	0.28
Ash	1.39	1.20	1.44	1.22	0.07	0.60	0.58	0.55	0.58	(0.09)
Nitrogen	0.31	0.30	0.31	0.31	(0.03)	0.20	0.22	0.20	0.20	0.01
Lignin (by difference)	1.83	1.87	1.97	1.78	(0.32)	6.48	6.40	6.52	6.64	0.19
Quantitative extractions (Constituents as % of oven-dry matter)										
Ligroin	3.49	3.51	3.63	3.66	0.04	1.66	1.64	1.61	1.55	0.04
Ether	1.62	1.70	1.55	1.64	0.04	0.61	0.59	0.64	0.63	0.03
Chloroform	2.05	2.22	2.02	2.11	0.09	0.96	0.75	0.82	0.83	0.11
Ethyl acetate	1.00	0.93	0.93	0.97		0.50	0.59	0.49	0.51	
Acetone	4.27	4.49	4.28	4.13		4.00	3.83	4.28	4.05	
Ethyl alcohol	13.83	13.46	13.73	12.67		10.23	9.86	9.57	9.67	

* *Necessary difference between classes for a 5% level of significance.*

( ) *No significant differences.*

† *Single determination only.*

Discussion

The series of analyses to which the wheats were subjected accounts for about 95% of the constituents of the dry matter of the leaves, provided that the last three nitrogen fractions are calculated as protein. The procedure separates the constituents into a number of main fractions and examines some of these in more detail. The determinations are empirical and the names given to the various fractions must be interpreted with due respect to the method of determination.

In the main series of analyses it became increasingly difficult to obtain duplicates that checked, owing to the cumulative effect of small errors. These effects were more noticeable in the seedling series in which the amount of material left for the later determinations was small. In the series of quantitative extractions the analyses also became progressively less accurate. This was not the result of cumulative errors, since these were avoided by resampling between extractions. As far as could be determined the errors are associated with the higher boiling points of the solvents used in the last half of the series. High boiling solvents emphasize differences in size, length and shape of the vapor tubes of the Soxhlet extractors with the result that differences in the rates of siphoning of the extractors are increased. With ethyl acetate, acetone and alcohol, the errors introduced by variations in siphoning rate were greater than the differences between the various wheats.

The four classes of wheat proved to be very similar in composition at each of the two stages of growth. Nevertheless the determinations were sufficiently accurate to show that differences occurred between the wheats in a large number of the fractions. As might be expected, the differences between the wheats at the seedling and mature stage were very considerable.

In examining the data for possible relations between chemical constitution and rust reaction, the nature of seedling reaction and mature-plant reaction must be borne clearly in mind. The former is really misnamed: it is a characteristic of the plant throughout its whole life. Mature-plant reaction, on the other hand, is well named. It represents a type of resistance which develops as the plant matures and which is superimposed upon the plant's original seedling reaction.

The combinations of wheat classes used in these studies and the fact that they were examined at two stages of growth, make it possible to examine the data with a view to determining whether quantitative differences in any of the fractions are related to either seedling reaction or mature-plant reaction. If a difference in the amount of any fraction is responsible for a difference in seedling reaction, then at both stages of growth the classes RR and RS probably should contain either more or less of that fraction than the classes SR and SS. On the other hand, if a difference in the amount of any fraction is responsible for a difference in mature-plant reaction, then there should be no differences between the classes at the seedling stage, and at the mature stage the classes RR and SR should contain either more or less than the classes RS and SS.

TABLE II

ANALYSES OF HYBRID LINES OF WHEAT, DIFFERING IN THEIR RUST REACTIONS, AT TWO STAGES OF GROWTH

Determination	Wheat class, seedling				Necessary difference*	Wheat class, mature				Necessary difference*
	RR	RS	SR	SS		RR	RS	SR	SS	
Dry matter										
Air-dry matter as % of green wt.	18.40	18.77	18.48	18.53	(0.64)	24.81	24.41	23.94	23.61	0.96
Oven-dry matter as % of A.D. matter	92.24	92.40	92.28	91.49	0.08	94.17	93.84	93.79	93.87	0.12
Oven-dry matter as % of green wt.	19.95	20.31	20.03	20.25	—	26.35	25.80	25.52	25.15	—
Main series of analyses (Constituents as % of oven-dry matter)										
Total ash	14.13	13.58	14.12	13.65	0.09	8.18	8.22	8.32	8.27	0.05
Total nitrogen	6.19	6.15	6.13	6.23	0.03	2.25	2.35	2.30	2.24	0.02
Ether extract	5.09	5.24	5.23	5.40	0.09	2.28	2.21	2.26	2.13	0.03
Cold water extract	37.05	36.40	37.43	36.61	0.17	22.32	21.96	21.89	21.47	0.27
Ash	10.32	10.23	10.04	10.38	(0.76)	6.05	6.12	6.16	6.31	0.08
Nitrogen	1.51	1.40	1.48	1.48	0.03	0.90	0.93	0.94	0.91	0.03
Total reducing compounds	3.94	3.76	4.20	4.04	0.08	5.67	5.60	5.22	5.26	0.07
Reducing sugars	1.16	0.96	1.32	1.21	0.07	4.02	3.97	3.69	3.70	0.13
Invert sugar	6.63	7.07	7.01	6.83	0.15	3.31	2.75	3.09	2.91	0.11
Hot water extract	3.11	3.04	3.21	3.16	(0.33)	2.40	2.50	2.49	2.38	0.09
Ash	0.98	0.96	1.07	1.01	(0.12)	0.60	0.65	0.63	0.64	0.02
Nitrogen	0.16	0.15	0.15	0.16	(0.02)	0.07	0.08	0.07	0.07	0.01
Total reducing compounds	0.55	0.52	0.57	0.56	0.05	0.62	0.65	0.61	0.62	0.01
Reducing sugars	0.17	0.13	0.16†	0.17	—	0.37	0.37	0.36	0.36	(0.04)
Invert sugar	0.07	0.07	0.08†	0.08	—	0.08	0.07	0.09	0.08	(0.03)
Alcohol extract	3.37	3.57	3.36	3.41	0.11	2.31	2.25	2.39	2.21	0.05
Hydrochloric acid extract	34.29	34.49	33.66	34.13	0.25	33.28	33.28	33.04	33.37	(0.76)
Nitrogen	3.75	3.74	3.69	3.77	0.05	0.89	0.93	0.88	0.88	0.02
Hemicellulose	10.95	11.00	10.55	10.81	0.07	22.07	22.14	22.19	22.48	0.23
Sulphuric acid extract	12.21	12.19	12.07	12.36	(0.52)	28.78	28.51	29.41	29.47	0.62
Nitrogen	0.33	0.31	0.34	0.33	0.01	0.12	0.12	0.12	0.11	(0.02)
Cellulose	8.83	8.85	8.74	8.93	0.19	24.32	24.11	24.91	25.10	0.47
Residue	5.12	4.94	5.30	4.89	(0.49)	8.32	8.82	8.29	8.47	0.28
Ash	1.39	1.20	1.44	1.22	0.07	0.60	0.58	0.55	0.58	(0.09)
Nitrogen	0.31	0.30	0.31	0.31	(0.03)	0.20	0.22	0.20	0.20	0.01
Lignin (by difference)	1.83	1.87	1.97	1.78	(0.32)	6.48	6.40	6.52	6.64	0.19
Quantitative extractions (Constituents as % of oven-dry matter)										
Ligroin	3.49	3.51	3.63	3.66	0.04	1.66	1.64	1.61	1.55	0.04
Ether	1.62	1.70	1.55	1.64	0.04	0.61	0.59	0.64	0.63	0.03
Chloroform	2.05	2.22	2.02	2.11	0.09	0.96	0.75	0.82	0.83	0.11
Ethyl acetate	1.00	0.93	0.93	0.97		0.50	0.59	0.49	0.51	
Acetone	4.27	4.49	4.28	4.13		4.00	3.83	4.28	4.05	
Ethyl alcohol	13.83	13.46	13.73	12.67		10.23	9.86	9.57	9.67	

* *Necessary difference between classes for a 5% level of significance.*

( ) *No significant differences.*

† *Single determination only.*

Discussion

The series of analyses to which the wheats were subjected accounts for about 95% of the constituents of the dry matter of the leaves, provided that the last three nitrogen fractions are calculated as protein. The procedure separates the constituents into a number of main fractions and examines some of these in more detail. The determinations are empirical and the names given to the various fractions must be interpreted with due respect to the method of determination.

In the main series of analyses it became increasingly difficult to obtain duplicates that checked, owing to the cumulative effect of small errors. These effects were more noticeable in the seedling series in which the amount of material left for the later determinations was small. In the series of quantitative extractions the analyses also became progressively less accurate. This was not the result of cumulative errors, since these were avoided by resampling between extractions. As far as could be determined the errors are associated with the higher boiling points of the solvents used in the last half of the series. High boiling solvents emphasize differences in size, length and shape of the vapor tubes of the Soxhlet extractors with the result that differences in the rates of siphoning of the extractors are increased. With ethyl acetate, acetone and alcohol, the errors introduced by variations in siphoning rate were greater than the differences between the various wheats.

The four classes of wheat proved to be very similar in composition at each of the two stages of growth. Nevertheless the determinations were sufficiently accurate to show that differences occurred between the wheats in a large number of the fractions. As might be expected, the differences between the wheats at the seedling and mature stage were very considerable.

In examining the data for possible relations between chemical constitution and rust reaction, the nature of seedling reaction and mature-plant reaction must be borne clearly in mind. The former is really misnamed: it is a characteristic of the plant throughout its whole life. Mature-plant reaction, on the other hand, is well named. It represents a type of resistance which develops as the plant matures and which is superimposed upon the plant's original seedling reaction.

The combinations of wheat classes used in these studies and the fact that they were examined at two stages of growth, make it possible to examine the data with a view to determining whether quantitative differences in any of the fractions are related to either seedling reaction or mature-plant reaction. If a difference in the amount of any fraction is responsible for a difference in seedling reaction, then at both stages of growth the classes RR and RS probably should contain either more or less of that fraction than the classes SR and SS. On the other hand, if a difference in the amount of any fraction is responsible for a difference in mature-plant reaction, then there should be no differences between the classes at the seedling stage, and at the mature stage the classes RR and SR should contain either more or less than the classes RS and SS.

Careful examination of the data has failed to reveal any relation between any fraction and either type of rust reaction. This was to be expected in view of the fact that the fractions determined represent major groups of plant constituents rather than individual compounds. Nevertheless, the investigation, though its results are negative, forms the first step in a logical attack upon the rust problem by the forces of analytical chemistry, and paves the way for more detailed, difficult, and time-consuming investigations of the possible relations of individual constituents of the wheat leaf to rust reaction.

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A MICROSCOPICAL STUDY OF INFECTION OF THE ROOTS OF STRAWBERRY AND TOBACCO SEEDLINGS BY MICRO-ORGANISMS OF THE SOIL[1]

By A. A. Hildebrand[2] and L. W. Koch[3]

Abstract

Similarity of organisms encountered in studies of black root of strawberry and of tobacco, respectively, carried out contemporaneously but independently, suggested the co-operative investigation, the results of which are embodied in this paper. Strawberry and tobacco seedlings growing (i) in seed-bed muck heavily infested with *Thielaviopsis basicola* (Berk.) Ferraris, and other organisms known to be pathogenic on tobacco, (ii) in soil from a commercial plantation where strawberry root rot had occurred in severe and typical form, and (iii) in greenhouse compost soil, were examined microscopically daily, commencing a few hours after germination and continuing throughout a period of four weeks. Organisms observed definitely within root tissues of both hosts included the "phycomycetous mycorrhizal" fungus, *T. basicola* (observed in plants grown in muck only), *Rhizoctonia* (*Solani* and endophytic orchid types), forms of *Pythium*, *Asterocystis* (*Olpidiaster*), certain unidentified fungi, a minute filamentous alga and nematodes. Organisms observed on the surface of roots included representatives of the genera *Cylindrocarpon* (*Ramularia*), *Fusarium*, *Helminthosporium*, *Sphaeropsis*, and *Cephalothecium*. The sequence of appearance, percentage occurrence, and parasitic capabilities of certain of the organisms varied in roots grown in the different soils. Because of early infection by, and ultimate almost universal occurrence of, the phycomycetous mycorrhizal fungus this organism received especial attention. Evidence based on certain morphological differences suggests the occurrence of strains of this organism. Of interest, too, is an alga invading living root tissue.

From observations not limited alone to the examination of diseased roots of strawberry and tobacco, the authors are led to conclude, (1) that a root rot as it occurs in nature is extremely complex even in cases where a primary causal agent is recognized, and (2) that fungi representative of comparatively few groups or genera are "common factors" in root-rot complexes of different host plants.

The technique described offers distinct advantages in that it permits a study of the sequence and severity of infection by the organisms involved in a root-rot complex; it reveals the occurrence of obligate parasites the presence of which would never be detected by the soil-plating, the Cholodny, or the tissue-isolation methods; and it is readily adaptable to the study of other root-rot complexes.

Introductory

In the course of investigations on black root of strawberry (8) and of tobacco (11), respectively, carried out simultaneously but independently during the past two years at the St. Catharines laboratory, microscopical examinations of affected roots, supplemented by large numbers of isolations, have shown that representatives of the same genera or groups of fungi, as well as nematodes, are commonly associated with both diseases as they occur typically in nature on their respective hosts. This seemed of interest in view of the fact that in black root rot of tobacco, an annual plant, a single pathogen,

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namely, *Thielaviopsis basicola*, is regarded as the primary causal agent of the disease, whereas in black root rot of strawberry, a perennial plant, recent investigations and observations by Strong and Strong (15), Berkely and Lauder-Thomson (1), Truscott (16), Hildebrand (8) and Hastings (7) have shown that different primary parasitic organisms may attack the roots. In the light of our present knowledge, however, it is impossible to select a specific organism as the causal agent of the disease.

Of further interest is the fact that while both diseases may cause serious damage to older roots, their attack on roots in the primary condition is especially severe. A diseased condition and paucity of finer absorbing laterals are outstanding characteristics of black root rot of strawberries and it is well known that black root rot of tobacco may cause serious loss of seedlings. The similarity of the organisms encountered in these characteristically different root rots suggested to the present authors the advisability of a co-operative investigation on certain phases of the problem at least. The further fact that the attack of the two diseases on roots in the primary condition is especially severe, suggested the study of the roots of seedlings as the logical starting point. In the present paper are embodied, therefore, the results of an intensive microscopical study of the complete root systems of a large number of seedlings of tobacco and strawberry, employing a clearing and staining technique which renders possible a study of infection by, and recognition of, micro-organisms residual in the soils of plantations and seedbeds in which the respective diseases had occurred in severe and typical form.

Material and Methods

Two different soil types were used in the present study. The first, a muck, such as is more usually used in tobacco seedbeds, was chosen because it had already produced several crops of plants affected with black root rot and because, in addition to *T. basicola*, it was known to be heavily infested with the "phycomycetous mycorrhizal" fungus (8, 10, 11, 12, 16) and with representatives of other genera of fungi known to be pathogenic on tobacco (11). The second, an ordinary clay loam, for convenience hereinafter referred to as "strawberry-root-rot soil", was obtained from a commercial plantation where strawberry root rot had occurred for several seasons. It has already been reported in a previous publication (8) that when strawberry runners were trained into this soil, they developed into "degenerate" plants exhibiting typical symptoms of black root rot. Tobacco seed, variety Judy's Pride, and strawberry seed, variety Nich Omer, were sown in the greenhouse in 14-inch clay saucers containing, respectively, sterilized and non-sterilized soil of the two types. Both kinds of seeds were also planted in saucers of sterilized sand and in addition strawberry seeds were planted in greenhouse compost soil. To insure uniform stands, a weighed quantity of each kind of seed was sown in the respective saucers.

Commencing a few hours after the radicles had started to emerge from the testa and continuing for a period of four weeks, at the end of which time the

roots had developed laterals of the third and fourth order, seedlings of both strawberry and tobacco were removed daily from the soil and examined microscopically, a few unstained, but the majority cleared and stained using lacto-phenol with acid fuchsin added, according to the technique more recently employed by Truscott (16), Hildebrand (8) and Koch (11). The results which follow are based on the critical study of the complete root systems of over 350 seedlings of tobacco and 400 of strawberry.

Strawberry and Tobacco Seedlings Grown in Muck

Organisms Observed within Root Tissues

Strawberry and tobacco seeds planted in muck showed first signs of germination on the seventh and fifth days, respectively, following planting. Within 24 hours after germination, during which time radicles had developed to lengths ranging from 2–4 mm., specimens were first obtained for microscopical examination. The organisms observed within the tissues of these newly formed radicles and of the later formed roots included the following:

Thielaviopsis basicola

Four of ten radicles of strawberry, which could not possibly have been in contact with the soil for a period exceeding 18–24 hours showed infection by *T. basicola* (Plate I, Fig. 6). Reference to Table II will show that infection of tobacco seedlings by *T. basicola* was first observed on the fourth day after germination. Examination of Table I will show that of a total of 60 strawberry seedlings examined during the first 11 days 15% showed infection by *T. basicola* while 20% of the 79 tobacco seedlings examined during the same period showed infection by this organism. During the longer period, *i.e.*, from the 11th to the 30th day, 2.5% of the 40 strawberry seedlings and 75% of the 120 tobacco seedlings examined, showed infection by *T. basicola*. Of interest in this connection is the 55% increase in infection on tobacco, the naturally susceptible host, as contrasted with the 13% decrease in infection on strawberry, a host which would appear to be susceptible to infection by this organism only during early stages of development of the seedling.

In regard to the parasitism of *T. basicola* on the two hosts it was noted in the case of strawberry that although there was definite intracellular penetration there was no apparent necrosis of invaded cells, and infections did not progress beyond an incipient stage. In tobacco, on the other hand, the fungus was an aggressive parasite, producing the well known symptoms of black root rot.

Rhizoctonia

Infection by two distinct types of *Rhizoctonia*, namely, the *Solani* and orchid types (8, 11, 16) was observed on both strawberry and tobacco. During the first 11 days 17.5% of the tobacco seedlings examined showed infection by *Rhizoctonia* of the *Solani* type. During the subsequent 11- to 30-day period, infection by representatives of this type had decreased to 13.5%. Similarly in the case of strawberry during the two corresponding periods

TABLE I

PERCENTAGE OCCURRENCE OF MICRO-ORGANISMS IN AND ON ROOTS OF SEEDLINGS OF TOBACCO AND STRAWBERRY, COMPUTED FROM RESULTS OF DAILY MICROSCOPICAL EXAMINATION DURING 30-DAY PERIOD AFTER GERMINATION

	Tobacco	Strawberry	Tobacco	Strawberry	Tobacco	Strawberry	Tobacco	Strawberry	Strawberry
	Seed-bed muck				Strawberry-root-rot soil				Compost soil
Number of roots examined	79	60	120	40	50	100	45	70	12
Age of roots in days	1–11	1–11	11–30	11–30	1–11	1–11	11–22	11–22	1–21
Organisms within root tissues									
Phycomycetous mycorrhizal fungus	15.0	31.6	92.5	75.0	10.0	14.0	55.0	74.3	20.0
T. basicola	20.0	15.0	75.0	2.5	0.0	0.0	0.0	0.0	0.0
Rhizoctonia, Solani type	17.5	11.6	13.5	2.5	2.0	1.0	3.0	0.0	5.0
Rhizoctonia, orchid type	0.0	6.6	6.2	2.5	10.0	13.0	22.0	20.7	5.8
Pythium	16.2	0.0	8.2	2.5	12.0	16.0	9.3	12.8	3.3
Asterocystis	0.0	0.0	2.0	0.0	0.0	0.0	2.0	2.8	0.0
Alga	Frequent	Frequent	Frequent	Frequent	Frequent	Frequent	Frequent	Frequent	0.0
Unidentified	3.7	8.3	6.0	2.5	14.0	16.0	12.0	4.3	21.6
Nematodes	0.0	0.0	5.1	0.0	8.0	27.0	35.5	33.3	5.0
Organisms on surface of roots									
Cylindrocarpon	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Fusarium	1.0	1.6	3.0	2.5	3.0	0.0	3.0	1.4	0.0
Helminthosporium	0.0	0.0	0.0	0.0	5.0	2.0	7.0	1.4	0.0
Sphaeropsis	1.0	0.0	1.0	0.0	0.0	0.0	2.0	0.0	0.0
Cephalothecium	1.0	0.0	1.5	0.0	1.0	0.0	1.0	0.0	1.0
Unidentified	3.0	3.3	2.0	0.0	11.0	11.0	3.0	2.8	5.0
Roots free from infection	11.2	38.3	6.0	22.5	18.0	30.0	6.0	8.5	44.1

there was a decrease in infection from 11.6% to 2.5%. During the first 11 days tobacco was free from infection by the orchid type of *Rhizoctonia*, whereas the older seedlings examined showed 6.2% infection. This is in contrast with the recorded observations on strawberry. Younger seedlings of the latter host showed 6.6% infection, which decreased to 2.5% in the older seedlings.

Regarding the parasitism of *Rhizoctonia* on the two hosts it was noted in connection with the *Solani* type that penetration was more usually effected at or near the ground level and resulted in considerable damping-off in tobacco but scarcely any in strawberry. In both hosts, however, a sufficient number of clear-cut cases of tip infections resulting in necrosis and disorganiza-

TABLE II

SEQUENCE OF INFECTION OF ROOTS OF TOBACCO AND STRAWBERRY SEEDLINGS BY MICRO-ORGANISMS RESIDUAL IN THREE DIFFERENT TYPES OF SOIL

Days after germination	Muck		Strawberry root-rot soil		Greenhouse compost
	Tobacco	Strawberry	Tobacco	Strawberry	Strawberry
1		*T. basicola* *R. Solani*			
2				*R. Solani* Nematodes	Orchid Rhiz.
3			Orchid Rhiz.	Orchid Rhiz.	*R. Solani* *Pythium* Nematodes
4	*Pythium* *T. basicola*			*Pythium*	
5	*R. Solani*	Phyco. Mycor. Orchid Rhiz.		Phyco. Mycor. Orchid Rhiz.	
6	Phyco. Mycor. Orchid Rhiz.	*Pythium*			
7			Alga		Phyco. Mycor. Orchid Rhiz.
8			Nematodes		
10	Alga		*R. Solani*		
11			*Pythium*	Alga	
12		Alga	Phyco. Mycor. Orchid Rhiz.		
14	Nematodes				
15	Orchid Rhiz.				
25	*Asterocystis*		*Asterocystis*		
28				*Asterocystis*	

tion of tissue (Plate I, Fig. 1) was observed to indicate that representatives of the *Solani* type are capable of causing definite root rot as distinct from damping-off.

In both hosts, where infection by the endophytic orchid type of *Rhizoctonia* was observed, it was noted that even in heavily infected roots where many of the cells were completely filled with the mycelial complex of the fungus (Plate I, Figs. 4 and 5), there was relatively little discoloration or disorganization of cells or tissue invaded by the fungus. The lack of virulence displayed by the orchid type of *Rhizoctonia* was in marked contrast to that exhibited by members of the *Solani* group.

Pythium

Only 2.5% of 40 strawberry seedlings examined showed infection by *Pythium*, whereas 16.2% of the 79 younger and 8.2% of the 120 older tobacco seedlings showed infection by representatives of this genus. While in strawberry *Pythium* was relatively unimportant, nevertheless, in tobacco the observations indicated that members of the genus are important pathogens as regards both damping-off and root rot.

The "phycomycetous mycorrhizal" fungus

During the first 11 days 15% of tobacco and 31% of strawberry seedlings showed infection by the "phycomycetous mycorrhizal" fungus. By the end of 30 days, however, 92% of the tobacco and 75% of the strawberry seedlings showed heavy infection. Later, in this soil, it proved impossible to find root systems of either strawberry or tobacco free from invasion by this fungus.

Asterocystis (Olpidiaster)

A few of the older tobacco seedlings examined revealed the presence of *Asterocystis* but this obligate parasite was not observed in strawberry seedlings less than 30 days old.

Alga

Both in tobacco and in strawberry there was frequently observed in individual cortical cells, at or near the ground level, an organism which in stained material somewhat resembled a nematode both in size and general appearance, except that it was multiseptate. Examination of fresh material showed, however, that it was a chlorophyll-bearing organism, a member of the filamentous type of blue-green algae. As yet no effort has been made to identify this alga definitely with a view to determining its taxonomic position. Examination of a large number of roots invaded by this alga indicates that penetration is effected by mechanical means. Following direct penetration through the cell wall, the organism coils within the cell (Plate I, Figs 2 and 3). In no case has necrosis been correlated with the presence of the organism when it occurs alone. That it is not restricted to the soils used in the present experiments but is probably widely distributed in nature, is suggested by the fact that Koch has noted its presence in tobacco seedlings obtained from commercial seedbeds. An evaluation of the significance of the presence of

this organism either as a possible primary parasite, or as an agent providing an infection court for other organisms, has not yet been made. Meanwhile it must be regarded as a possible factor in the root-rot complex.

Nematodes

As reference to Table I will show, nematodes were not observed in the roots of strawberry and tobacco and only in about 5% of the older seedlings of the latter host.

Unidentified fungi

Of a total of 100 younger and older seedlings of strawberry examined, an average of 5.4% were found to be infected by fungi which could not be identified. The same was true for the 199 tobacco seedlings examined, the occurrence of unidentified fungi being slightly lower, namely an average of 4.8%. Since previous investigators (1, 15, 16) have shown certain imperfect fungi to be pathogenic on strawberry roots it is possible, particularly in the case of strawberry, that important pathogens may be included in the unidentified fungi, but since their mycelium within the tissues lacks the distinguishing characteristics necessary for even a generic diagnosis, it is impossible to evaluate the significance of their occurrence in a study of this kind.

Organisms Observed on the Surface of Roots

In addition to the organisms observed within the root tissues, certain fungi including representatives of the genera *Cylindrocarpon (Ramularia)*, *Fusarium*, *Helminthosporium*, *Sphaeropsis*, and *Cephalothecium* were also noted on the surface of roots. These fungi were for the most part identified by their conidia. The latter were frequently observed germinating on the surface of the roots but in no case was penetration by the germ tubes definitely noted.

Strawberry and Tobacco Seedlings Grown in "Strawberry-root-rot" Soil

Strawberry and tobacco seeds planted in the "strawberry-root-rot" soil showed the first signs of germination on the ninth and sixth days, respectively, after planting. As in the case of seeds planted in muck, examination of the seedlings was begun within 24 hours subsequent to germination. While the organisms observed within the roots of the seedlings of both hosts growing in strawberry-root-rot soil were more or less identical with those found in the roots of seedlings grown in muck—with the outstanding exception of *T. basicola* —nevertheless the percentage of occurrence of these organisms and the sequence of infection by them (Tables I and II), showed considerable variation in the two types of soil. The organisms which were definitely identified are recorded below in the same order as in the preceding section.

Rhizoctonia

While both the *Solani* and orchid types of *Rhizoctonia* were observed in both hosts, infection by the first-mentioned type occurred much less frequently. Not more than 3% of the tobacco seedlings, nor more than 1% of the strawberry seedlings showed infection by this fungus, though considerable surface

mycelium was observed. These percentages are in marked contrast to those calculated for infection in the muck soil. These observations would suggest that *R. Solani* as one of the components in the root-rot complex may vary markedly with soil type. Percentage infection by the orchid type of *Rhizoctonia* varied from 10–13 in the younger seedlings of tobacco and strawberry respectively, to 22 and 20 in older seedlings of the two hosts. Examination of Table I will show that these percentages are much higher than those recorded for seedlings grown in muck.

The observations recorded in the preceding section regarding the parasitism of the two types of *Rhizoctonia* on seedlings grown in muck confirm these made in connection with the seedlings grown in the strawberry-root-rot soil. It will be noted, however, that the relative occurrence of the two types of this organism varies with soil type.

Pythium

Whereas only 2.5% of 40 strawberry seedlings grown in muck showed infection by *Pythium*, 16% of 100 younger and 12.8% of 70 older strawberry seedlings grown in the root-rot soil showed infection by representatives of this genus. In the case of tobacco, however, there was a slight decrease from 24.4% of the 119 seedlings grown in muck to 21.3% of the 95 grown in the root-rot soil.

The examination of seedlings grown in root-rot soil indicates that *Pythium* is an important primary pathogen on both hosts. In many cases where root extremities were rotting off, *Pythium* alone could be found in the disintegrating tissues of the affected roots. As in the case of *Rhizoctonia*, while *Pythium* is undoubtedly the cause of damping-off, more especially in the case of tobacco, nevertheless, sufficient tip infections were observed to show that this fungus is responsible for definite root rot.

The "phycomycetous mycorrhizal" fungus

During the first 11 days 10% of the tobacco and 14% of the strawberry seedlings showed infection by the "phycomycetous mycorrhizal" fungus. These percentages by the end of 30 days had increased to 55 and 74.3. Later, as in the case of seedlings grown in muck, it was impossible to find root systems of either host free from invasion by this fungus.

Asterocystis (Olpidiaster)

Approximately the same percentage of *Asterocystis* was observed in the older roots of tobacco seedlings grown in the strawberry-root-rot soil as in those grown in muck. In the case of strawberry, whereas this fungus was not observed at all in roots of seedlings grown in muck, it was noted in 2.8% of older seedlings grown in the root-rot soil.

Alga

In the seedlings of both hosts grown in the strawberry-root-rot soil the alga to which reference has already been made in the preceding section was frequently observed.

Nematodes

One of the most striking differences in the occurrence of micro-organisms in the two types of soil was that noted in regard to nematodes. Whereas in muck these organisms were entirely lacking in the younger seedlings of both hosts and were observed in only 5% of the tobacco seedlings up to 30 days old, on the other hand, in the root-rot soil they were present in 8% of the tobacco and 27% of the strawberry seedlings up to 11 days old, these percentages increasing to 35.5 and 33.3 in older seedlings. Hildebrand (8) and Koch (11) have already suggested the importance of nematodes in the root-rot complex. In the present study they were observed alone and in association with various fungi. In many cases where they occurred alone necrosis of invaded cells indicated primary parasitism on the part of these organisms, while in certain cases, discoloration and at least partial disintegration of cells contiguous to those invaded suggested some such "action in advance" as, according to Goodey (6), was observed by Quanjer in the case of *Anguillulina dipsaci*, a parasite of potatoes and various other plants. Even though definite proof is lacking as to the primary parasitic capabilities of these organisms, in any case they must provide by mechanical means or otherwise, ideal infection courts for facultative parasites which otherwise could not establish themselves within the host. Where nematodes and fungi were found together, even in incipient infections it was impossible to determine which of the organisms had gained entry first.

The observations in general in regard to nematodes suggest that these organisms are an important factor in the root-rot complex, more particularly, perhaps, as it obtains in the case of strawberry.

Unidentified fungi

As reference to Table I will show, the percentage of roots in which unidentified fungi were observed, was higher for seedlings grown in strawberry-root-rot soil than for those grown in seed-bed muck. Thus, of a total of 170 strawberry seedlings examined, an average of 10.1% showed infection by unidentified fungi as compared with an average of 5.4% in strawberry seedlings grown in seed-bed muck. Of 95 tobacco seedlings grown in the strawberry-root-rot soil, an average of 13.0% showed infection by unidentified fungi, whereas an average of only 4.8% of those grown in seed-bed muck were infected by fungi which could not be identified.

Organisms observed on the surface of root tissues

In regard to organisms which were observed on the surface of root systems, what has been said for seedlings grown in muck also holds true for seedlings grown in the root-rot soil.

Strawberry Seedlings Grown in Greenhouse Compost Soil

Of one hundred and twenty 1- to 21-day-old strawberry seedlings grown in greenhouse compost soil, 44% were found to be healthy and the rest remained relatively freer from infection than those grown in the muck and the root-rot soil. The "phycomycetous mycorrhizal" fungus

the seedlings examined. Primary infection by the fungus was first noted on the seventh day, as compared with the fifth day in the other two types of soil. Infection by the two types of *Rhizoctonia* was also appreciably less in the compost soil, the *Solani* and orchid types being observed in only 5 and 5.8% of the seedlings, respectively. The alga which had been noted so frequently in seedlings grown in muck and in the root-rot soil was not observed at all in those grown in the compost soil. Nematodes were noted in 5% of the seedlings and they differed in type from those observed in the seedlings from the other two soils. Very few organisms were noted on the surface of the roots of the seedlings grown in the compost soil.

The "Phycomycetous Mycorrhizal" Fungus in Tobacco and Strawberry

Of all the organisms that could be definitely identified within the tissues of the roots of tobacco and strawberry, the phycomycetous mycorrhizal fungus was the most conspicuous in appearance and the most consistent in occurrence. The widespread distribution of this organism in strawberry, together with suggestions as to its possible significance as a factor in the root-rot complex has been pointed out by O'Brien and McNaughton (12), Truscott (16), and Hildebrand (8). More recently Koch (11) has shown that the same or a closely similar organism is present in the roots of tobacco, in the thalli of certain liverworts, and in the stems and leaves of certain mosses. Opinions vary as to the significance of the presence of this fungus in regard to pathological conditions in the strawberry. O'Brien and McNaughton (12), and Truscott (16) conclude that this endophyte is not only a definite parasite but is an extremely important pathogen of the strawberry. In regard to tobacco the evidence adduced by Koch (11) indicates that the fungus possesses parasitic capability on this host.

In view of the fact that the endophyte in question seemed to indicate not only different pathogenic capabilities on tobacco and strawberry, but also exhibited certain morphologic differences on the two hosts, it was decided to study the organism more critically with a view to obtaining additional information, whatever its nature, concerning this obligate parasite which, never having been grown in pure culture, cannot be studied directly.

Infection and development stages

In regard to infection by, and developmental stages of, the endophyte, reference to Table III will show that initial infection was first noted in tobacco grown in muck, six days, and in strawberry five days, after germination. In root-rot soil initial infection of tobacco was noted 13 days, and in strawberry five days, after germination. In greenhouse compost soil initial infection of the latter host was first noted seven days after germination. In tobacco, arbuscules appeared two to three days after initial infection while in strawberry they appeared three to four days after initial infection in all three soil types. In this connection it is of interest to note

that whereas Reed and Fremont (14), in their study of endotroph mycorrhiza in the roots of citrus, observed arbuscules in certain cas only, particularly "where cells appear to be in complete physiological equili rium (trees regularly receiving a complete fertilizer)", in the present studi arbuscules were especially abundant in both hosts regardless either of ho vigor or of soil type. In tobacco, both in muck and in root-rot soil, vesicl appeared 15 days after germination. In strawberry, on the other hand, th appearance of vesicles seemed correlated with soil type, since in seedling grown in muck, in root-rot soil and in compost they appeared in 9, 14 and 1 days respectively after germination of seeds. The "sporangiole" stage wa not observed within the four-week period of intensive examination of seedlings

TABLE III

INITIAL INFECTION BY, AND DEVELOPMENTAL STAGES OF, THE "PHYCOMYCETOUS MYCORRHIZAL" FUNGUS IN THE ROOTS OF TOBACCO AND STRAWBERRY SEEDLINGS, GROWN IN THREE DIFFERENT SOILS

First appearance of	In tobacco		In strawberry		
	In muck, days*	In strawberry-root-rot soil, days	In muck, days	In strawberry-root-rot soil, days	In compost, days
Infection	6*	13	5	5	7
Arbuscules	8	16	8	9	10
Vesicles	15	15	9	14	19
Necrosis of host tissue	17	18	—	—	—

* *After germination.*

In regard to the vesicles, the present investigations shed no further light as to the true nature of these structures, that is, whether they are resting bodies, storage organs or sporangia. Peyronel (13) in 1923, and Van der Pijl (17) in 1934, recorded the presence of spores in the vesicles of the endophyte. The critical examination of hundreds of vesicles in both stained and unstained material of different ages has revealed within these bodies the presence of numerous small oil globules which in untreated material suggest the delimitation of the contents into spore-like bodies, but which, as Truscott (16) has already pointed out, when treated in lacto-phenol, coalesce into one large globule.

Ontogenetic Relations

Early in the investigation it was casually noted in the case of tobacco that infection by the endophyte occurred anywhere from the root tip to the root-stem transition, whereas, in strawberry, infection either in the immediate region of the root tip or the root-stem transition was of rare occurrence. This seemed of interest in that there was suggested in the case of strawberry a correlation between infection and ontogeny of the root.

A critical examination of 100 roots of each host up to 21 days old was made to compare the distribution of infection especially in relation to root tip and root-stem transition. The results obtained are shown graphically in Fig. 1. It will be observed from an examination of Fig. 1, A, that in tobacco not only

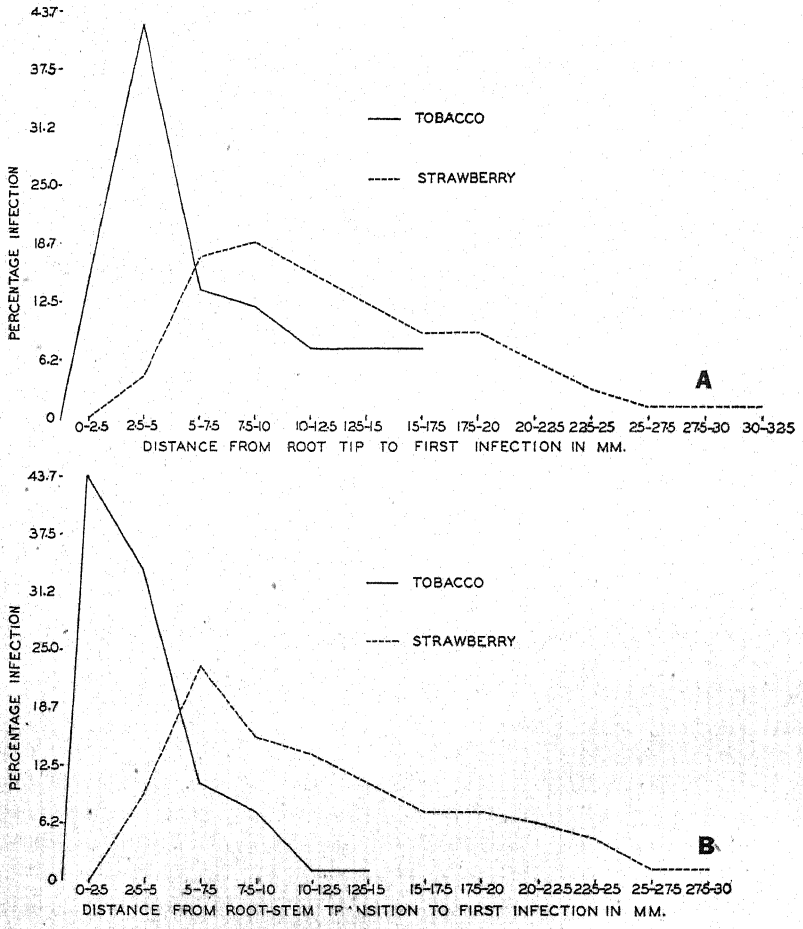

FIG. 1, A and B. *Curves showing percentage occurrence of most distal and most proximal infections in relation to root tip and root-stem transition.*

did tip infection occur but that the maximum of the most distal infections, 41%, occurred within the range of 2.5–5 mm. from the root tip and that only 7% of the most distal infections occurred within the range of 15.0–17.5 mm. from the root tip. On the other hand, in the case of strawberry, only a single case of tip infection was observed and the maximum of the most distal infections, 18.7%, occurred within the range of 7.5–10.0 mm. from the root tip, with a small percentage of the most distal infections occurring within the range of 30.0–32.5 mm. from the root tip.

With regard to the most proximal infections, examination of Fig. 1, B, wil show that in tobacco the maximum of such infections, 43.7%, occurred withi the range of 0–2.5 mm. from the root-stem transition and that only 1% o these infections occurred within the range of 12.5–15 mm. from the root stem transition. On the other hand, in strawberry, the maximum of th most proximal infections, 23%, occurred within the range of 5.0–7.5 mm from the root-stem transition, while only 1% occurred within the range o 27.5–30.0 mm. Between these two limits most proximal infection decreased in direct proportion to the increasing distance from the root-stem transition.

From the above results it would appear, then, that in strawberry in the regions of both the root tip and the root-stem transition, tissues of seedlings are resistant to infection by this fungus. In tobacco, such regions free from infection do not exist. The reason for the apparent immunity of certain regions of the roots in the case of strawberry is not known. It is possible, however, that some such correlation exists between ontogeny and infection, as Fitzpatrick (5) has demonstrated in the case of infection of peach leaves by *Taphrina deformans.*

It should be pointed out that in the region intermediate between the most distal and the most proximal infections heavy infection usually occurred in the roots of both hosts.

Morphological differences between the endophytes in the two hosts

Early in the present investigation it became apparent that certain morphological differences existed between the organism noted in tobacco and that observed in strawberry. In strawberry, when a strand of extramatrical mycelium comes in contact with the surface of the root an irregularly shaped appressorium-like enlargement of the mycelium very often develops (Plate II, Fig. 1). From the appressorium-like enlargement a wedge-like hypha develops, pushing its way between the epidermal cells (Plate II, Figs. 1 and 2). From this advancing and penetrating hypha, mycelium develops which ramifies in the outer cortical cells (Plate III, Fig. 4). Coiling of mycelium in these earlier invaded cells was not observed. On the other hand, in tobacco, when an extramatrical strand of mycelium comes in contact with the surface of the root, in many cases such a strand continues along the surface, at intervals producing infection hyphae which penetrate the epidermal cells and which, instead of ramifying in all directions, as in the case of strawberry, produce characteristic coils in the invaded cells (Plate III, Fig. 3). From these coils, some of which are also formed in the outer cortical cells as well as in the epidermal cells, hyphae ramify in the underlying cortical tissues.

In the deeper cortical tissues of the strawberry invaded by this fungus a characteristic paralleling of intercellular mycelium was noted, which was lacking in the corresponding tissues of tobacco. A further difference was noted in the degree of septation of the mycelium. In tobacco, septa were of relatively much more frequent occurrence than in strawberry (Plate III,

Figs. 3 and 4). Short papilla-like protuberances are frequently characteristic of the mycelium of the strawberry endophyte (Plate II, Fig. 3) but were not noted in the case of the tobacco organism.

The arbuscules of the organism in strawberry (Plate II, Fig. 4) and tobacco are essentially similar but marked differences were noted in the case of the vesicles. The latter in strawberry are essentially more or less oval (Plate III, Fig. 2) and, as Truscott (16) has already pointed out, "tend to be somewhat distorted in the intercellular spaces". In tobacco the vesicles are typically sphaeroidal (Plate III, Fig. 1; Plate II, Fig. 5) though in this host as well, irregularly-shaped vesicles are sometimes observed. Six hundred and seventy-seven vesicles in tobacco were examined critically as to their shape. Of this number 474 (70%) were sphaeroidal, 125 (18%) were more or less oval (in this respect resembling more closely those observed in strawberry), and 78 (11%) were irregular in shape. The same number of vesicles were examined in strawberry and more than 95% were more or less oval in shape, the remainder being irregular, conforming to the configurations of the intercellular spaces. Intracellular vesicles were of much more frequent occurrence in tobacco than in strawberry.

Regarding the parasitism of this fungus on strawberry and tobacco, it should be pointed out that while in the latter host considerable necrosis of tissue could be correlated with the presence of the endophyte, in strawberry, on the other hand, necrosis was seldom observed though depletion of starch in invaded cells was a common occurrence. In the cortical region of tobacco, necrosis of invaded cells was consistently observed in cases where intracellular vesicles were present (Plate II, Fig. 6).

In summarizing the data presented in the foregoing paragraphs it is believed that sufficient evidence has been presented to warrant the view that the fungus which has hitherto been referred to as the "phycomycetous mycorrhizal" fungus is not a specific entity but consists of strains which can be distinguished more particularly on a basis of morphological differences.

Discussion

As Eaton and King (4) in their study of the cotton root-rot fungus have recently pointed out, "progress in soil microbiology has been handicapped by the lack of methods which give a true picture of the numbers, activities and relationships of the organisms under field conditions". It is believed that the technique described in the present investigations promises not only to assist in overcoming the handicaps referred to above, but in permitting the direct observation of sequence and relative degree of infection, also contributes materially to our knowledge of two outstandingly important root-rot diseases. While the present studies were conducted in the greenhouse there is no reason why the methods employed could not be modified for studies of root rots of the same or other host plants as they actually occur in nature. The method undoubtedly has its limitations since, for example, the organisms which can be observed are for the most part restricted to those which are pathogenic,

and thus it is impossible to evaluate the relationship, antagonistic or otherwise, existing between these pathogenic organisms and the numerous other micro-organisms of the soil. Information regarding these relationships may be obtained, however, by supplementing the technique described in the present paper with the method devised by Cholodny (2) and used subsequently with some measure of success by Jensen (9), Eaton and King (4) and Demeter and Mossel (3).

Of interest in the present investigations is the fact that in the two characteristic types of root rot, that is, one in which a chief primary causal organism is recognized and the other in which are undoubtedly a number of pathogenic organisms, the sequence and relationships of which are not yet clearly understood, there is a surprising similarity in regard to the organisms encountered. It may be mentioned here that incidental to the present studies, the roots of a number of other host plants affected with root rot, including tomato, lettuce and tulip have been examined microscopically and the same complex picture involving infection by the same or very similar organisms was observed. The constant association of similar organisms in root rots of widely different hosts would indicate that there is a certain rather limited microfauna and microflora concerned with root rots generally. The sequence of infection by the various organisms associated in the complex and the evaluation of their relationship not only to one another but also to the other micro-organisms concerned in the microbiological equilibrium of the soil remains for further research.

In the body of the present paper considerable emphasis has been placed on the "phycomycetous mycorrhizal" fungus. It is believed, however, that such emphasis is warranted in view of the fact that this fungus is so universally present in the roots of the two host plants under investigation. An intensive study of this fungus as it occurs in these two hosts has revealed certain consistent morphological differences which point strongly to the occurrence of strains of the organism. There is the possibility, however, that such differences as have been observed may be due to the effect of the host on the fungus.

The technique described in the present paper seems of especial value for the reasons that (1) it is a quick, effective method readily adaptable to the study of root rots in general, (2) it permits a study of the sequence of infection by the organisms involved in a root-rot complex from the earliest stages of root development, and, (3) it reveals the occurrence within the root tissues of obligate parasites, the presence of which would never be detected by the soil plating, tissue isolation, or Cholodny methods.

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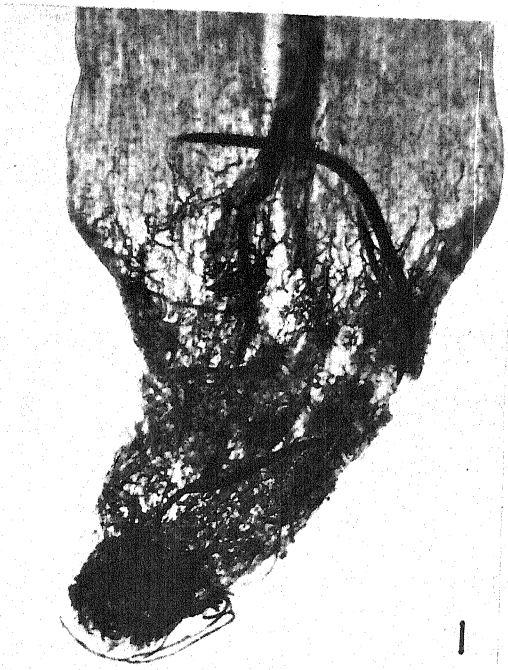
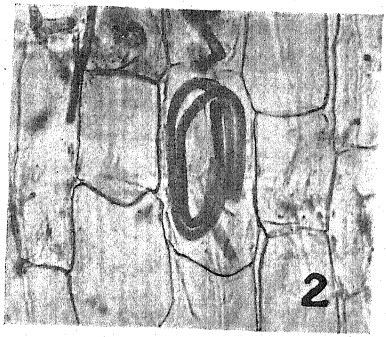
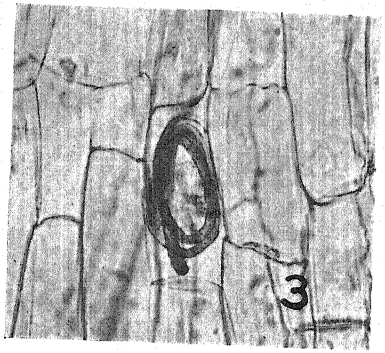
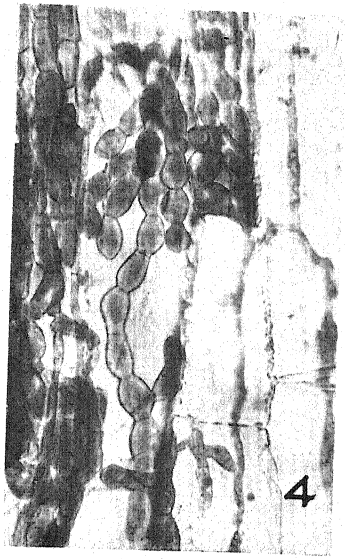








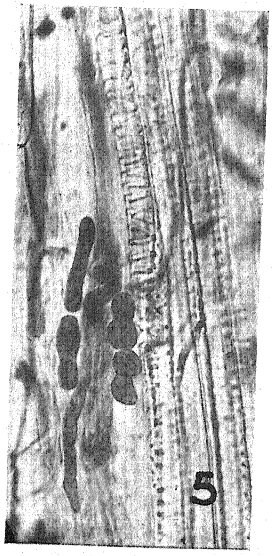
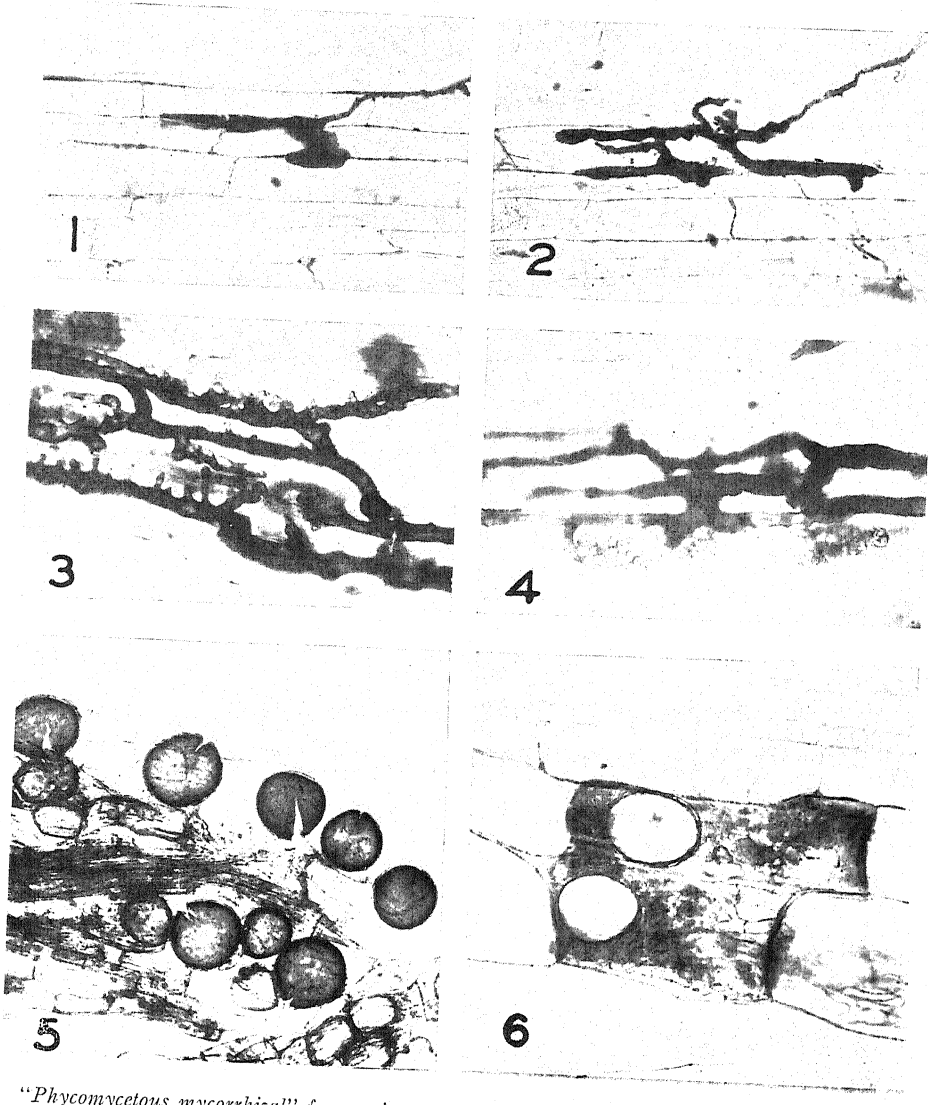
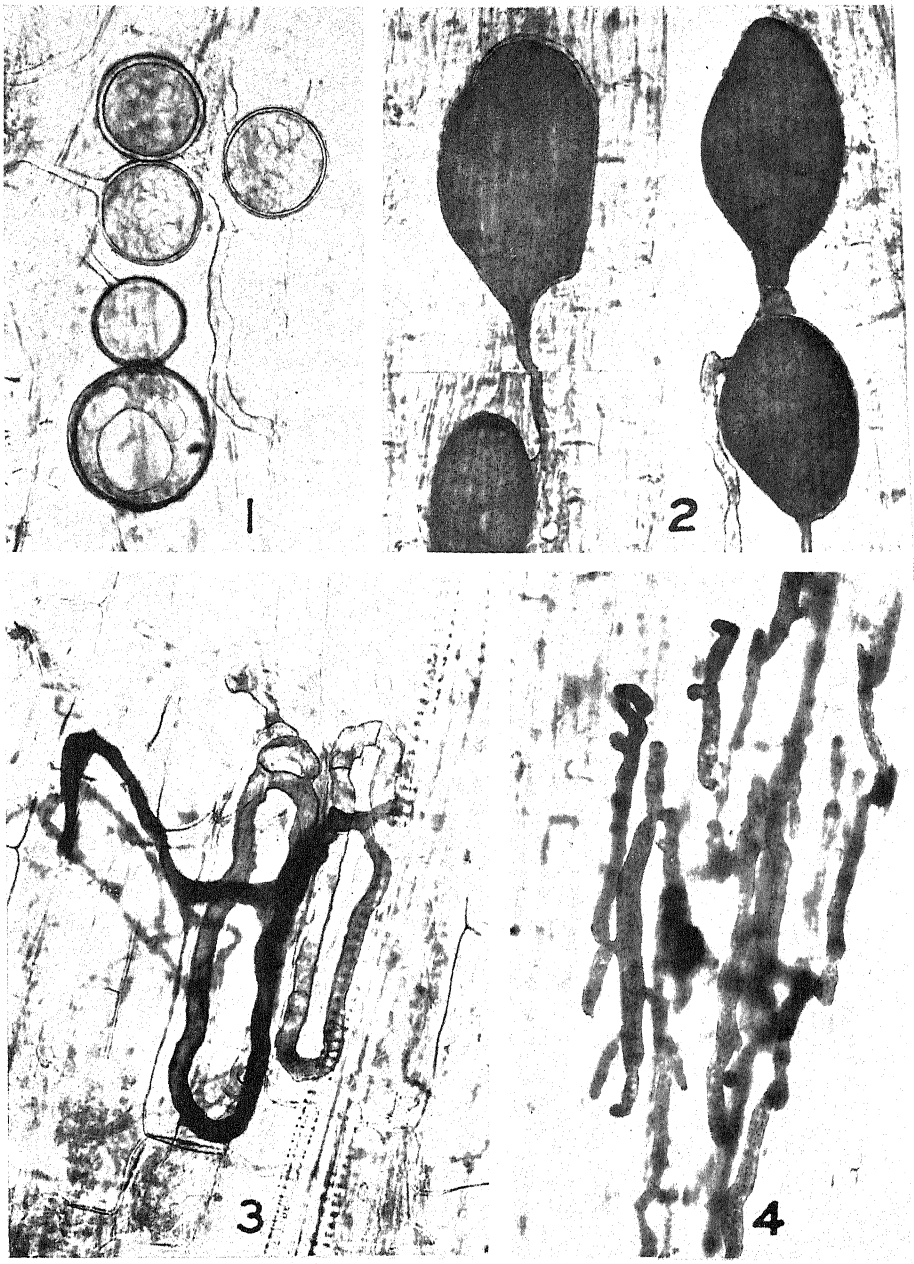


FIG. 1. *Tip of rootlet of strawberry seedling infected with Rhizoctonia Solani. Note presence also of a few nematodes* (× 70). FIGS. 2 AND 3. *Alga in outer cortical cells of rootlets of strawberry and tobacco, respectively* (× 360). FIGS. 4 AND 5. *Orchid type of Rhizoctonia infecting cortical cells of rootlets of strawberry and tobacco, respectively.* (×360). FIG. 6. *Thielaviopsis basicola infecting outer cortical cells of rootlet of strawberry seedling grown in naturally infested seed-bed muck* (× 360).



"Phycomycetous mycorrhizal" fungus in roots of strawberry (Figs 1–4) and of tobacco (Figs. 5 and 6). (All × 340). FIG. 1. *Early stage of infection showing strand of extramatrical mycelium and appressorium-like enlargement from which wedge-like penetrating hypha is pushing its way (towards the left) between epidermal cells.* FIG. 2. *Later stage of infection. Note wedge-like penetrating hypha at lower left.* FIG. 4. *Later stage of infection showing formation of first arbuscules.* FIG. 3. *Showing papilla-like protuberances commonly observed on mycelium of strawberry endophyte.* FIG. 5. *Tobacco rootlet showing late stage of infection. Note typically sphaeroidal vesicles.* FIG. 6. *Invaded cells of tobacco rootlet showing necrosis correlated with presence of intracellular vesicles.*



Photomicrographs showing outstanding morphological differences between the "phycomycetous mycorrhizal" endophyte as observed in the rootlets of tobacco (Figs. 1 and 3) and of strawberry (Figs. 2 and 4). FIG. 1. *Typical sphaeroidal vesicles of tobacco endophyte (× 340).* FIG. 2. *Oval-shaped vesicles typical of strawberry endophyte (× 360).* FIG. 3. *Early stage of infection by tobacco endophyte showing characteristic coiling of mycelium in outer cortical cells. Note also presence of septa (× 340).* FIG. 4. *Corresponding stage of infection by strawberry endophyte showing mycelium ramifying in outer cortical tissues without coiling. Note absence of septa (× 340).*

PEACH CANKER INVESTIGATIONS

II. INFECTION STUDIES[1]

By R. S. Willison[2]

Abstract

Two species of *Valsa* have been isolated more or less consistently from cankers of various ages, and from "die-back" twigs on the peach. In culture, one species, identified as *Valsa leucostoma* (Pers.) Fr., is hair-brown and has small dark pycnidia exuding cirri when mature. On the host, its stroma is compact in texture, contains no host cells and is delimited beneath by a black zone of carbonized fungal and host cells. Ascospores of *V. leucostoma* measure 10–17 by 2–4.5 μ. The other species, which has been assigned to *V. cincta* Fr., is whitish to olive buff in culture and has large light-colored pycnidia containing, though rarely exuding, spores. On the host, the stroma of *V. cincta* is comparatively loose in texture, contains host cells and is delimited from the cortex of the host by a thin, black zone, sometimes only marginal. Ascospores of *V. cincta* measure 14–28 by 4–7 μ. In both species, the pycnospores range from 5 to 10 μ in length and 1 to 2 μ in width. These organisms, along with *Sclerotinia fructicola* (Wint.) Rehm., were used in series of infection experiments at frequent intervals over a period of two years. Similar series of checks were also provided. Periodical observations and measurements furnished detailed case histories of all wounds concerned. *V. cincta* was found to be a virulent wound-parasite, able not only to infect freshly made wounds during the late autumn, winter and spring, but also to give rise to perennial cankers. Infection with this organism rarely occurred during June, July and August. *V. leucostoma* proved in these experiments to be almost, if not quite, incapable of initiating cankers on the peach. *S. fructicola* parasitized the tissues of branches and produced considerable necrosis during the first three weeks after inoculation during the growing season. Subsequently the lesions proceeded to heal. The degree of infection and the amount of resultant necrosis in wounds inoculated with *S. fructicola* during the dormant season were dependent upon the conditions of temperature and humidity then prevailing. *S. fructicola*, while capable of inducing lesions on the stem, cannot be regarded as the cause of typical peach canker. Some of the factors influencing infection by the three organisms mentioned above are briefly discussed.

In the first paper of this series (55), a number of factors contributing to the occurrence of canker on peach trees in the Niagara Peninsula were discussed and it was pointed out that the disease was due to the activities of a fungous wound-parasite. The present article deals with infection experiments concerning three fungi, two of which have been considered (23, 24, 28, 35, 44, 51) as the causal organisms of peach canker and of certain forms of "die-back". To avoid confusion, attention is directed at present to that phase of the disease manifested as cankers on larger branches, leaving for future consideration the phase affecting twigs, and its relation to the general subject of "die-back".

Much has been written in recent years concerning canker, gummosis, "die-back", "apoplexy" and kindred ailments of the stone fruit trees. However, the literature on the subject is to a large extent controversial, partly because of the tendency to seek a common cause for these troubles on the

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various hosts, and partly because these terms are sometimes employed to denote the same disease and sometimes entirely different ones. The following conclusions reached by certain investigators will serve to illustrate the existing confusion as well as the necessity both for further fundamental work and for the correlation and evaluation of existing information. Pfeiffer (40) attributes gummosis of peach, cherry, plum and apricot in Saxony to unfavorable cultural conditions rather than to bacterial invasion. Shilberszky (46) considers the dying-off of stone fruit trees to be due to the obstruction of conducting elements by tyloses and gummification associated with "abnormal enzymatic activity", to the physiological effects of severe frosts, especially those of late winter and spring, to unfavorable soil conditions, or to deep planting. In a later paper, (47) he extends the causes to include wounding, incompatible stocks and parasitic infection, especially by *Clasterosporium carpophilum* and *Sclerotinia cinerea*. At the same time, he claims to have refuted the view that *Cytospora* spp. and *Valsa* spp. are implicated in gummosis except in trees already weakened from other causes. Czarnecki (14), Van der Meer (52), Dufrénoy (16, 17), and Joëssel (29) suggest that infection by *Verticillium* spp. is an important factor in the dying-off of apricot and peach trees. However, Joëssel (29) and Joëssel and Bordas (30) consider that the disease is frequently associated with injuries and that *Verticillium* is only one of several causes. Faes and Staehelin (21) have not so far obtained evidence of the pathogenicity of species of *Clasterosporium*, *Monilia*, *Fusarium*, and *Verticillium* in the dying-off of apricots. On the other hand, Rives (41, 42) has demonstrated that "apoplexy" of apricot may be caused by bacterial organisms. While Curzi (13) submits evidence of wilting of peaches due to infection by *Phytophthora* spp., *Fusicoccum persicae* is cited as a possible cause of twig cankers of peach by Overholts (39) and *Nectria cinnabarina*, of "die-back" of apricot by Dowson (15).

The problem of gummosis, canker, "die-back", dying-off and/or "apoplexy" of stone fruits is evidently one of considerable complexity, presenting numerous phases, one or more of which have been investigated by each of the workers referred to above. At this point, it might be well to reiterate that the causal factors applicable to the disease complex on one host are not necessarily applicable to that on closely related hosts. This idea has already been broached by Miss Cayley (6) in connection with the behavior of *Cytospora* sp. and *Diaporthe perniciosa* on stone fruits, and later by Chabrolin (8) and Joëssel and Bordas (30) in reference to "apoplexy" of apricots. While peaches and apricots, on the one hand, and plums and cherries on the other, seem to have much in common in this regard, specific differences should not be overlooked. Regional variations in climate, soil and so forth should be considered, as they doubtless play their part in determining the factor or combination of factors operative in various districts. Thus, in order to be able to diagnose particular cases adequately and to recommend suitable control measures, it would seem necessary to take into account all factors which might be involved.

The almost constant association of *Cytospora* spp. and of *Valsa* spp. with canker and "die-back" in stone fruits has led investigators to consider that members of these genera are the primary agents. Aderhold (1) was one of the first to prove that *Cytospora* spp. could become established in dead areas on cherry and thence parasitize healthy tissues. A little later, Rolfs (44) succeeded in infecting, by cross inoculation, peach, plum, cherry, wild cherry and apricot with strains of *Cytospora* and *Valsa* isolated from each of these hosts. However, the times and methods of his inoculations, and even the organism used, are not always clear. But other workers have not been satisfied that "die-back" and canker are primarily due to these organisms. Miss Cayley (6) demonstrated that *Diaporthe perniciosa*, though only weakly parasitic, was more vigorous than *Cytospora* spp. on stone fruits, with the exception of peach. After failing to induce typical lesions on plum with *Cytospora* spp., Wormald (57, 63) showed that *Pseudomonas Mors-Prunorum* was the cause of canker, and frequently of death, in plum trees, although the lesions were soon overrun with *Cytospora* sp. Wilson (56) described a similar bacterial canker on plum, cherry, apricot and peach. On the other hand, McCubbin (35) was able to induce cankers in the peach with an organism designated as *Valsa leucostoma*. He introduced pieces of the organism from cultures under a V-shaped flap of bark, and then covered the wound with cloth dipped in grafting wax. He also found that, by artificially freezing the tissue prior to inoculation, infection was more easily achieved and the resulting damage greater. This experiment, however, cannot be taken as proving anything more than the ability of the *Valsa* sp. to establish itself in dead tissue. Togashi (51) accomplished similar results by inoculating branches of both peach and apricot with *Leucostoma Persoonii* (Nitsch.) Togashi (syn. *Valsa leucostoma* (Pers.) Fr.) and with *Valsa japonica* Miyabe & Hemmi, by way of small cross slits cut with a scalpel hot enough to kill some of the adjacent tissue. He used spore suspensions as inoculum, covered the wounds with moist absorbent cotton and paraffin paper for five days, wetted the cotton daily, and afterwards painted the wound surface. Even with this method, he was unable to infect Terada plum and had only partial success with Golden Sugar plum. When a cold scalpel was used for making the incisions, infection did not occur even in peach (inoculated August 10, 1927). Unfortunately only one attempt was made with the cold scalpel, although the other method was employed in eight experiments between May 30 and November 14, 1927.

In a number of recent papers, considerable evidence has been presented to the effect that there is a marked seasonal variation in the ability of specific parasites to infect the woody parts of their host. In the case of wound-parasites, infection occurs most readily during the dormant season. Brooks and Moore (4) showed that *Stereum purpureum* can readily attack fresh wounds on plum at any time except June, July and August. Wormald (63) found *Pseudomonas Mors-Prunorum* infecting plum most effectively during the period from October to December but not at all from April to September.

Togashi (51) considered the critical period for infection of the peach with *Leucostoma Persoonii* and *Valsa japonica* to extend from the middle of August to the middle of November. Homma (26) reported similar results for *Camarosporium persicae* on peach and *Prunus mume*. Wilson (56) obtained more extensive bacterial cankers on plum and cherry by inoculating in late autumn and early spring, than he did in early autumn or late spring. Curzi (13) found that *Phytophthora* spp. gained entry to tissues during the dormant period, from October to spring. By way of contrast, Koch (31) demonstrated that the critical period for infection of plum twigs with *Dibotryon morbosum* is limited to May and June, the season of most vigorous growth. *D. morbosum*, however, exhibits a highly specialized type of parasitism which is quite distinct from that of the other organisms referred to.

The Fungi

In the course of the present investigations many isolations have been made from the internal tissues of cankers of various ages, and later, of monospores from fruiting bodies appearing on cankers and dead twigs. Predominant among the fungi cultured were two forms of *Cytospora*, easily distinguishable in culture, but separable only with difficulty on the host. In March 1931, with the discovery of perithecial stromata on the host, it was possible to identify these organisms as the imperfect stages of two species of *Valsa*. One agreed closely with descriptions, by Saccardo (45), Ellis and Everhart (18), and Togashi (50), of *V. leucostoma* (Pers.) Fr. The other has been tentatively designated as *V. cincta* Fr. as described by Saccardo (45) and Ellis and Everhart (18). Both species have been isolated frequently from cankers of several years' standing, though very rarely from the same canker. On the other hand, incipient cankers yielded *V. cincta* and *V. leucostoma* in the ratio of more than 20 : 1. Indeed, certain forms of incipient canker, notably lesions in the vicinity of buds, contained only *V. cincta*, except in a few cases where other organisms such as *Alternaria* spp. had become established in the dead tissue. *V. cincta* has also been isolated from incipient cankers on the apricot.

Valsa cincta and *V. leucostoma* are so similar in some respects, yet so dissimilar in others, that it seems desirable to point out the features by which each can be recognized. To facilitate comparison, descriptive data are presented in parallel in Table I. In this connection, it should be recognized that the object is not so much to present a complete description of these species of *Valsa* as to emphasize the salient differences between them. While the perithecial stromata of *V. cincta* and *V. leucostoma*, by virtue of differences in size of ascospores, are readily distinguishable on the host, the pycnidial stromata are not, and separation is impossible on the basis of measurements of pycnospores. However, if perithecial material is not available, as is often the case, the distinctive characteristics of the two organisms on artificial media provide a ready means either of recognition or of confirmation of identification by other means. Rolfs (44) identified the organisms with which he worked as *Valsa leucostoma* var. *rubescens*, and *V. leucostoma* var.

cincta, separated on the basis of slight cultural differences and of pycnospore sizes. His descriptions, indeed, support the idea that both were forms of *V. leucostoma*. However, his remark that "on peach and plum agar, white, hairy pustules occasionally formed, which were at first mistaken for perithecial stromata, but examination showed them to be pycnidial stromata" suggests that, at times, he did have *V. cincta*.

TABLE I

COMPARATIVE DESCRIPTIONS OF *Valsa cincta* FR. AND *V. leucostoma* (PERS.) FR., OCCURRING ON PEACH IN THE NIAGARA DISTRICT OF ONTARIO

	Valsa cincta Fr.	*Valsa leucostoma* (Pers.) Fr.
	On nutrient media	
Characteristics of cultures on potato-dextrose agar (5% dextrose) and on Leonian's* malt agar.	Surface of culture felty, white at first, later Tilleul Buff to Olive Buff (Ridgway), sometimes black in substrate. Very little aerial mycelium. Pycnidia large (1–3 mm. in diam.) white, felty, rarely if ever exuding cirri, though usually full of viable spores. (Plate I, Fig. 4.)	Surface of culture suede-like, at first white, later Hair-brown (Ridgway) or darker. Almost no aerial mycelium. Pycnidia small (1 mm. or less in diam.) rostrate, usually dark, exuding cirri when mature. (Plate I, Fig. 6.)
	Both organisms produce pycnidia more readily in cultures exposed to daylight than in those kept in the dark.	
	On the host	
Erumpent disc of stroma.	Muddy gray, usually circular, ostioles of perithecia, when present, usually peripheral, frequently surrounding the mouth of a pycnidium. (Plate I, Fig. 1.)	White at first, turning gray with age, frequently elliptical. Ostioles of perithecia, when present, central without regular order. (Plate I, Fig. 7.)
Stroma	Dark ectostromatic tissue not well developed. Comparatively loose in texture. Host cells prevalent in stroma *above* pycnidium and perithecia. Separated from subjacent cortical tissue of host by thin "black zone" of carbonized fungal and host cells, sometimes only marginal. (Plate I, Figs. 2 and 3.)	Considerable dark ectostromatic tissue beneath bark. Compact in texture. Host cells few or absent in stroma *above* pycnidium or perithecia. Separated from subjacent cortical cells of host by "black zone" of carbonized fungal and host cells, usually well developed. (Plate I, Figs. 5 and 8.)
Pycnidia	Multilocular with common ostiole. Definite wall, outer layers usually not carbonized. (Plate I, Fig. 3.)	Multilocular with common ostiole. Definite wall, outer layers sometimes carbonized on upper side of pycnidium, sometimes confluent with stroma. (Plate I, Fig. 5.)
Perithecia	Usually globose, sometimes compressed laterally. Circinate, frequently surrounding a pycnidium. Whole perithecial stroma usually on larger scale than in *V. leucostoma*. (Plate I, Fig. 2.)	Usually spheroidal, sometimes compressed longitudinally. Circinate, usually not in same stroma as pycnidium. (Plate I, Fig. 8.)
Pycnospores	(All spore measurements were made from fresh distilled water mounts from newly collected material.)	
	Allantoid 5.5–10 × 1–2 μ (mode 7 × 1.5 μ)	Allantoid 5.0–10 × 1–2 μ (mode 7 × 1.5 μ).
Ascospores	Allantoid 14–28 × 4–7 μ (occasionally longer). (Mode 20 × 5 μ).	Allantoid 10–17 × 2–4.5 μ (mode 13 × 3 μ).

* *Leonian's malt agar (32):— Primary potassium phosphate 2.4 gm.; magnesium sulphate 1.2 gm.; peptone 1.2 gm.; maltose 12.5 gm.; malt extract 12.5 gm.; agar 25.0 gm.; water 1000 cc.*

The reported pathogenicity of *Valsa leucostoma* under certain conditions (1, 6, 11, 22, 35, 44, 51, 53) and the frequency of the association of *Valsa cincta** with incipient cankers rendered it necessary to employ both species in inoculation studies, especially as preliminary trials had indicated a decided difference in their respective pathogenic capabilities. For several reasons, it was considered advisable also, to make parallel inoculations with *Sclerotinia fructicola* (Wint.) Rehm. (syn. *S. americana* (Worm.) Nort. & Ez.), the causal organism of brown rot of stone fruits in America. There are numerous references (2, 9, 10, 23, 24, 28, 33–36, 38, 43, 48, 49) to twig killing and bark injury due to this fungus, both on peach and on other stone fruits, while other members of the genus cause similar injury on pome and stone fruits in Europe and elsewhere (3, 12, 19, 20, 25, 27, 29, 37, 58–62). In the course of the present investigations many cases of twig blight and injury following both *Sclerotinia* blight of blossoms and brown rot of fruit have been observed. In 1913, failing to induce infection with *Valsa* spp., but succeeding with the brown rot organism, Jehle (28) came to the conclusion that *S. cinerea* (Bonn) Schroet. (as *S. fructicola* was then designated in America) was the cause of canker in peaches in New York State. Indeed the disease has often been referred to as "Brown rot canker". McClintock (33) observed the production of conidia of *S. fructicola* on stem lesions during the second season, while Manns and Adams (36) and Berkeley (2) reported the over-winter survival of blossom-blight cankers. On the other hand there has been considerable evidence, (5, 35) that *S. fructicola* is rarely, if ever, able to perpetuate cankers over a number of seasons. McClintock (34) in 1929 failed to discover any conidia on blossom-blight cankers in their second year. Roberts and Dunegan (43), after six years' experience, found that while the fungus remained alive over winter in a certain percentage of cankers each year, conidia were not produced (with one exception). They do not mention the enlargement of blossom-blight cankers during the dormant season following infection.

By means of repeated sub-culturing on Leonian's malt agar (32) a single strain of each of the organisms, *Valsa leucostoma*, *V. cincta* and *S. fructicola*, was used throughout the entire series of inoculations described below. The culture of *V. leucostoma* came from a single pycnospore but was indistinguishable from cultures of ascosporous origin. The culture of *V. cincta* originated from a single ascospore, while the strain of *S. fructicola* employed here was isolated from the wood of a branch beneath a brown rot mummy. Because of the differences in the time factor involved in the production of pycnidia, on one hand, and of hyphomycetous conidia on the other, the cultures of the *Valsa* spp. were from six to ten weeks old, while those of *S. fructicola* were approximately two weeks old when used for inoculation. To provide inoculum, spore suspensions of *V. leucostoma* and *S. fructicola* were easily obtained by shaking tube cultures covered with sterile distilled water, but in the case of *V. cincta*, the pycnidia had to be sliced with a sterile scalpel in order to liberate the pycnospores.

* *According to Schilberszky (47), Dr. A. Schellenberg considered Valsa cincta responsible for a die-back of peach. However, as all efforts to trace this reference have so far proved fruitless, it is impossible to give the citation here.*

Methods of Inoculation

Commencing August 24, 1931, successive series of inoculations were made, on trees of the Elberta variety, at fortnightly intervals until August 12, 1932. From then until October 6, 1933, the experiment was continued with the intervals ranging from one week, at critical times of the year, to one month. Thus the complete experiment covered slightly more than two calendar years and comprised 45 series. Each series consisted of four sets, one for each organism and one for check, distributed one set per tree. The technique was as far as possible uniform throughout, so that the variable factors were limited, in the main, to developmental phases of the host, and to meteorological conditions at the time of inoculation as well as to unavoidable differences in the trees themselves. The duration of the experiment through two complete growth cycles made it possible to check to a considerable extent the effects of the host phase at the time of inoculation. The comparatively large number of series tended both to provide a wide range of weather conditions and to minimize the effects of differences in host material. There remains, of course, the possibility of a decline in the virulence of the fungi as a result of repeated sub-culturing on artificial media. However, if any such loss of pathogenic ability occurred, it was so slight as to be imperceptible.

The procedure followed during inoculation was simple. For each set, five branches, usually of different ages and sizes, were surface sterilized for a distance of about four inches by brushing thoroughly with a tooth brush dipped in a 1 : 500 solution of mercuric chloride. These areas were then washed with a stream of freshly distilled water. A small piece of tissue, approximately one and a half by one-half inches, was then removed from each in such a manner that the long axis of the "blaze" was parallel to that of the branch. A cold scalpel, sterilized with mercuric chloride and wiped with a clean cloth, was used to make the incisions. Several drops of inoculum were then placed on the newly made wound by means of a medicine dropper. To approximate natural conditions as closely as possible, all wounds were left exposed to the weather. The obvious advantages of the absence of protective wrappings were considered to offset the possibly enhanced likelihood of chance infection and contamination. However, the non-inoculated wounds not only provided a check for this point but also served to indicate the behavior of uninfected wounds. During the first year (Series 1 to 25) each set also contained five pruning cuts, made without previous sterilization and inoculated in the same manner as the other wounds. In part of the second year (Series 32 to 45) a single slit, also about an inch and a half long but involving no removal of tissue, was substituted in each set for one of the "blazes" described above. All wounds in Series 31 were slits.

Observations

To preserve the identity of the wounds for purposes of observation, each one was labelled with an identifying tag and its location indicated on a diagrammatic sketch of the tree concerned. The original size of each wound

was recorded and its subsequent measurements ascertained from time to time. The latest observations and measurements were taken in October or November 1934 so that the history of the earliest series has been closely followed for three consecutive years and that of the latest for one year.

The data thus obtained are assembled in Figs. 1, 2 and 3. Fig. 1 indicates for each set of inoculations the percentage of wounds (i) increasing in size during the season in which inoculation took place, (ii) enlarging as active cankers during the dormant season of 1933–34 or at removal, (iii) enlarging

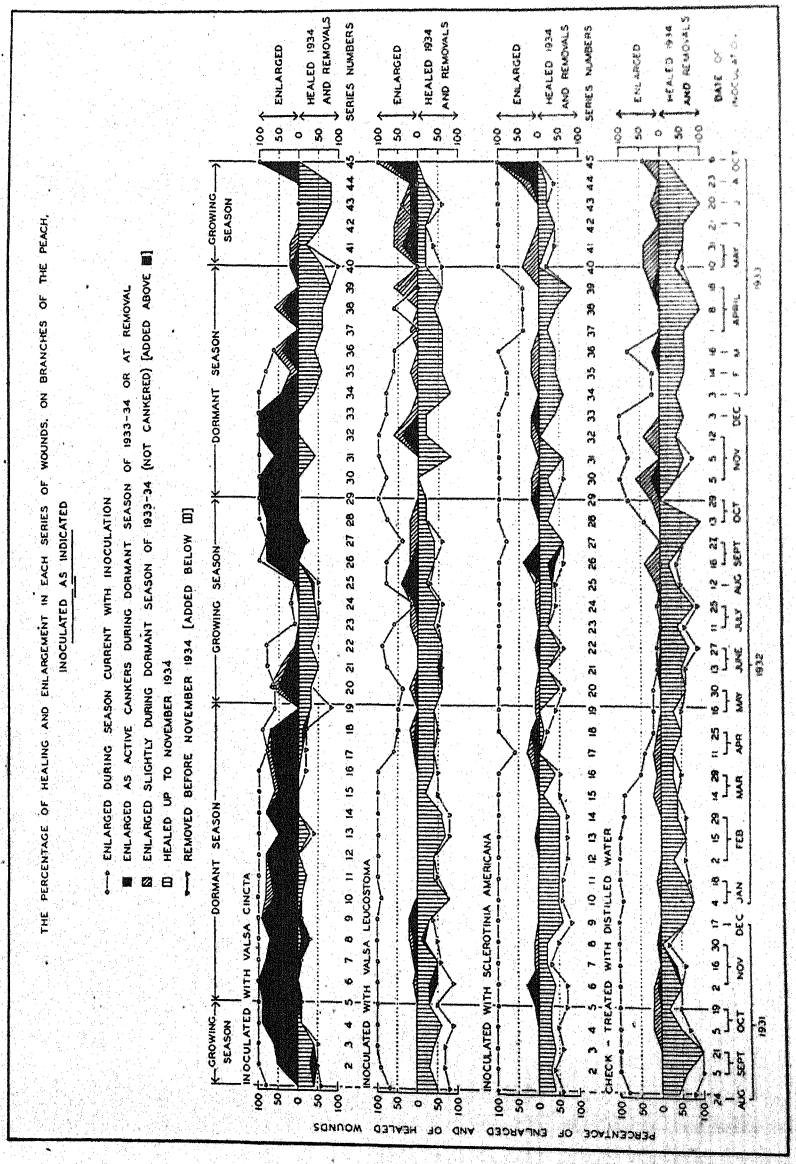

FIG. 1. *The percentages of wounds healed, enlarged and cankered in various series inoculated at different times of the year with Valsa cincta, V. leucostoma and Sclerotinia fructicola respectively, and checks.*

slightly, but not cankered during 1933–34, (iv) completely healed in November 1934, and (v) removed prior to November 1934. To avoid confusion, enlargements, except in the case of the earlier removals, appear above the base line while healed wounds and removals appear below. The measurements of wounds of the first type described in the preceding section are summarized in Figs. 2 and 3. Data relevant to the measurements of the pruning wounds have not been considered, in the first place, because it was thought advisable to deal only with comparable wounds throughout, and, in the second place,

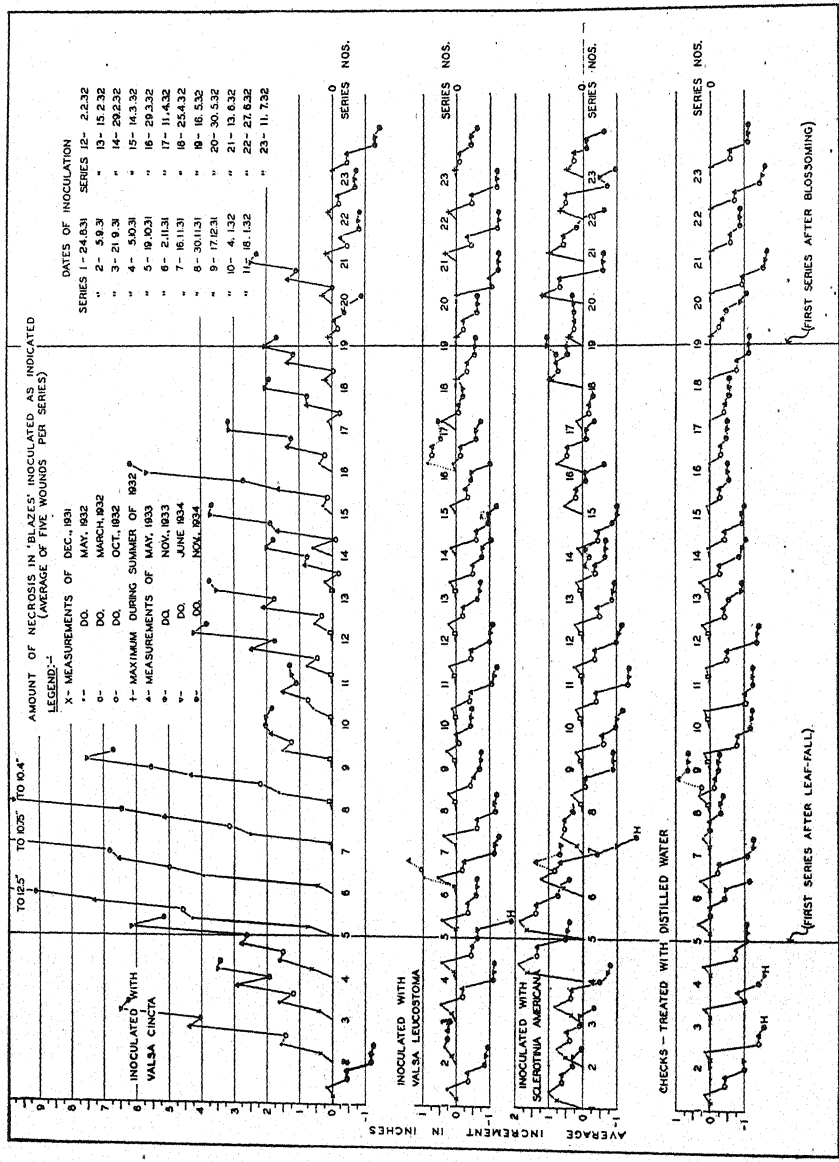

FIG. 2. *Records of progressive changes in the average size of wounds in each of Series 1 to 23, inoculated at different times of the year with V. cincta, V. leucostoma and S. fructicola respectively, and checks.*

because the behavior of the pruning wounds agreed in essence with that represented here. As necrosis advanced at a much greater rate at the ends of the wound parallel to the long axis of the branch, changes in the length of wounds were chosen as criteria for determining the progress of infection. Thus, each point on the various curves indicates the average length of a given set of wounds at the stated time, minus the average original length of the same wounds. As a consequence, the base lines represent average original lengths; positive ordinates, enlargements; and negative ordinates, decrease in size.

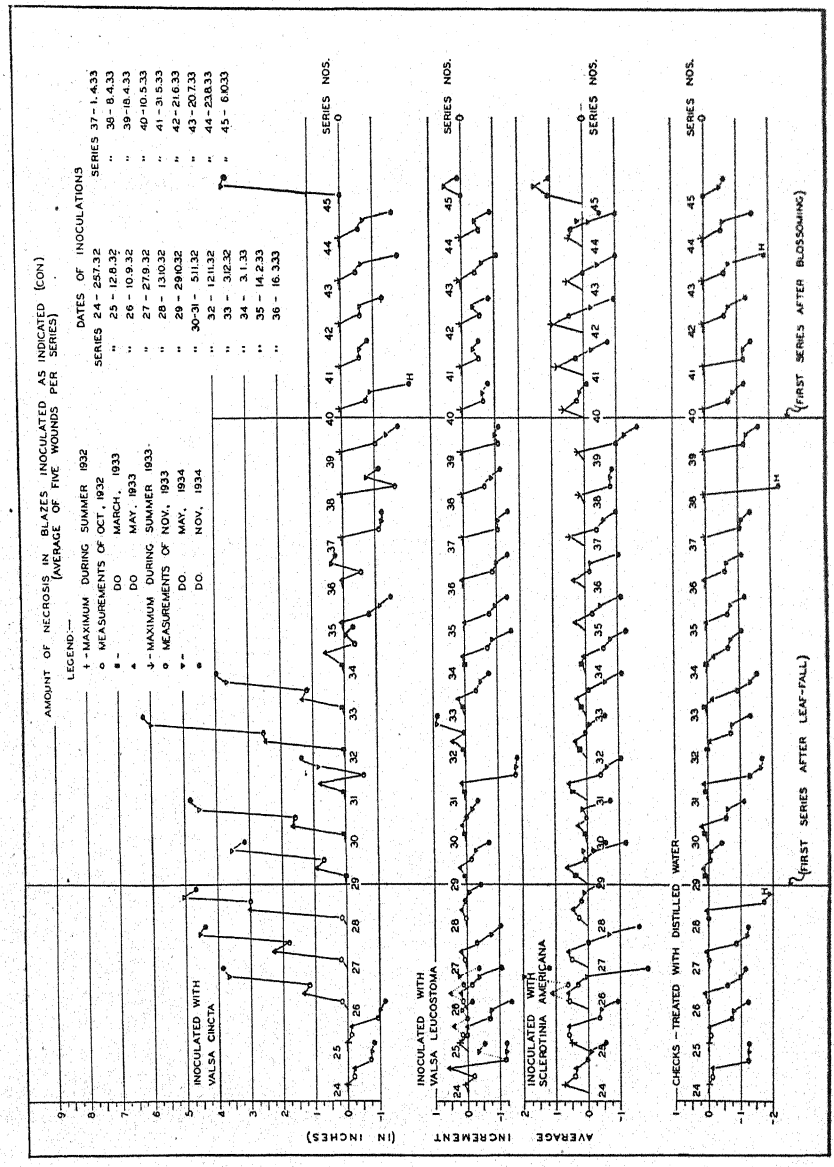

FIG. 3. *Records of progressive changes in the average size of wounds in each of Series 24 to 45, inoculated at different times of the year with V. cincta, V. leucostoma and S. fructicola respectively, and checks.*

For any one set of inoculations, the temporal sequence is shown, in a somewhat arbitrary fashion, by placing each successive measurement at a regular interval to the right of the preceding one. Consequently, the behavior of each set of inoculations is portrayed as a curve the upward tendency of which denotes necrosis and the downward tendency, healing. In those cases where, to avoid overlapping, the final reading or so has been omitted, that reading is the same as the last one which appears.

With these points in mind, even a cursory glance at these figures reveals fundamental differences in the reactions not only of the two *Valsa* species but also of *Sclerotinia fructicola*. Both the high percentage of active cankers developed in wounds inoculated at certain seasons of the year and their conspicuous and usually progressive enlargement are ample evidence of both the virulence of *V. cincta* under the conditions of the experiment and its ability to cause perennial cankers. On the other hand, *V. leucostoma* may be considered either as non-pathogenic or at best as an extremely weak wound-parasite. On the basis of active cankers produced, *S. fructicola* appears to rank with *V. leucostoma*, but the history of the wounds inoculated with the former organism reveals its strong though unsustained parasitic tendencies.

Discussion

V. cincta

It is of interest in connection with the present investigation, as close scrutiny of Figs. 1, 2 and 3 will show, that infection with *V. cincta* occurs most readily during the dormant season and for about six weeks before leaf fall, but only occasionally, if at all, during the height of the growing season, (compare Plate II, Figs. 1 and 2, Plate III, Figs. 6 and 7 with Plate III, Fig. 3). For a period in the late fall and early winter, determined largely by temperature conditions, *V. cincta* is able, shortly after inoculation, to cause visible damage, which progresses more or less steadily during the winter (Series 2–7, 26–28, Figs. 2 and 3). In the fall of 1931 this period lasted until November 25 during which time the mean daily temperature was usually well above 45° F. and sometimes as high as 60° F. The weather conditions prevailing for the corresponding season in 1932 were not so favorable for the fungus, as the mean daily temperature except for a few brief intervals, did not rise above 45° F. after October 29 (Series 29, Fig. 3). In the winter, when the mean temperature remained lower than 40° F., necrosis did not appear until the advent of warm weather in the spring (Series 8–13, 29–34, Figs. 2 and 3). This phenomenon is probably due to the inactivity of the host rather than to that of the fungus since there was a more or less steady decline in the initial amount of necrosis in successive sets of inoculations (Series 8–17, 32–35, Figs. 2 and 3). Moreover, Togashi (51) has demonstrated that internal temperatures in peach branches in winter frequently are considerably higher than the air temperature and often much above 32° F. Thus it may be argued that, in spite of generally unfavorable conditions and the consequent slowing down of fungal growth, opportunities are afforded during the winter months for spores to germinate and for the fungus to establish itself in the

host. It is logical, therefore, that the great extension of lesions during March and April is the result partly of the rapid growth of the fungus under the stimulus of more favorable temperatures and partly of the greater enzymatic activity of the host, inducing the discoloration which differentiates the infected from the healthy tissues. It is also reasonable to suppose that the amount of necrosis occurring at this time depends upon the foothold gained by the fungus during the winter, which in turn depends upon both the length of time and the conditions since inoculation. The discrepancy apparent in the behavior of *V. cincta* in Series 29 and 30 (Fig. 3) may in part be accounted for by the fact that, by reason of certain cultural practices (55), the trees involved were somewhat slower growing and hardier than those used in the subsequent series. In Series 31, (Fig. 3), as already noted, all wounds were slits which reacted somewhat differently than did the regular type of wound.

The failure of *V. cincta* to infect peach branches during a large part of the growing season may be explained by the interaction of three main factors, temperature, condition of the host, and the parasitic capabilities of the fungus. There is no doubt that this organism is unable to infect actively growing host tissue, but there is reason to believe that it can successfully attack mature, healthy tissues since invasion of callus in late atuumn has been observed in numerous older cankers, natural and artificial, during the present investigations. Consequently, during the period of active growth when callus is being rapidly formed, the canker decreases in size but the fungus is able to advance in the underlying woody tissues, hampered, it is true, by the presence of wound gum in the vessels and at times by temperatures adversely high (54). Now, if a freshly made wound is inoculated early in the growing season and conditions are suitable, *V. cincta* is sometimes able to invade the wood, although there may be little external evidence of infection at the time. Soon the growth of the fungus is retarded both by the deposition of wound gum in the host and by rising temperatures, while the development of the callus reduces the size of the wound. If, however, the fungus has succeeded in establishing itself, it invades the callus during the subsequent dormant season and a canker makes its appearance a year after infection actually occurred (Series 17, 18, 20, 21, 38, Figs. 2 and 3). The spring of 1932 was evidently favorable for infections of this sort while that of 1933 was not. The underlying reason is obscure but the shape of the curves indicates that the healing of new wounds proceeded at a greater rate in the latter year (compare all sets of Series 15–20 with those of 36–42, Figs. 2 and 3). It is also probable that the extreme heat combined with drought in the summer of 1933 did not permit appreciable growth of *V. cincta*, especially in recent infections. It would appear that, in the spring, there is a delicate balance between conditions favoring the growth of the fungus and promoting infection and those favoring the host and tending to prevent infection. During June, July and August the balance is decidedly in favor of the host (Series 1, 22–25, 42–44, Figs. 2 and 3). Then, the wound-gum barrier, which at other times can be penetrated by *V. cincta* (54) appears to be effective against the slowly growing

pathogen which fails to secure a foothold. In September and early October, before leaf fall, when prevailing temperatures are closer to the optimum for the fungus, infection is again possible. At that time, the host is able to form only a small amount of callus but wound gum still appears, so that the spread of the fungus through the tissues is less rapid than when infection occurs after leaf fall when the production of wound gum is negligible (54), and if low temperature is not a limiting factor (compare Series 2–4, 26, 27 with Series 5, 6 and 7, Figs. 2 and 3).

In those series where partial healing of the lesions during the growing season alternated with further enlargement during the dormant season (Series 2–4, 11–18, 20, 26–31 etc., Figs. 2 and 3), only a few of the inoculated branches became girdled. However, where the majority were killed by *V. cincta* (Series 5–8, 15, 32, Figs. 2 and 3) a progressive increase in the size of lesions was observed throughout the year, although the rate of increase was usually less during the growing season than during the dormant season. In many instances (*e.g.*, Series 8, Fig. 2) the canker spread down the girdled inoculated branch and produced a lesion on one side of the parent branch (Plate II, Fig. 1). Girdling was determined partly by the size of the branch involved and partly by the season during which infection occurred.

V. leucostoma

The curves (Figs. 2 and 3) illustrating the behavior of wounds inoculated with *V. leucostoma* are remarkably uniform in general character, and with the exception of greater initial increase in size in several instances, are very similar to corresponding curves for check wounds, (see also Plate II, Figs. 3, 4, 7 and 8). This may be taken as evidence that *V. leucostoma* is comparatively innocuous, although the initial spread suggests that this organism may be a mild irritant. Indeed, this fungus has been re-isolated six to eight months after inoculation from wounds showing little, if any, necrosis. It is true that a number of active cankers have appeared, but in any set except Series 45 (see Fig. 1) at least 60% of the wounds remained healthy (see also Figs. 2 and 3, Series 6, 24, 25, 26 where the dotted line represents the average of both cankered and healthy wounds and the solid lines, healthy wounds only). Moreover, in 62% of the sets no cankers were formed. Consequently, even conceding that *V. leucostoma* gave rise to all the cankers that did appear, which was not the case since some of them at least were proved by isolations to be due to sporadic infection by *V. cincta*, the ravages of the former organism cannot be considered as being very serious.

The evidence as presented above is regarded as sufficient to warrant the view that *V. leucostoma* is not capable of acting as a primary parasite causing peach canker, but not to eliminate altogether the possibility that *V. leucostoma* may, under other circumstances, infect the peach and become a factor in the production of cankers. The work of McCubbin (35) and Togashi (51) suggests that, given tissue in which it can become established as a saprophyte, this organism may subsequently become an active parasite and invade healthy

tissues. As *V. leucostoma* has been isolated from peach, and its fruiting bodies occur thereon, it can evidently live on that host. However, the fact remains that as a primary cause of canker it is of much less significance than *V. cincta*.

S. fructicola

The parasitism exhibited by *Sclerotinia fructicola* falls into a category quite distinct from that of *V. cincta*. Unlike the latter organism, *S. fructicola* attacks the bark and wood of peach most readily during the growing season and its spread through infected tissues is very rapid. Fom May to September, this organism accomplishes its work within a period of three weeks, at most, after inoculation. This period corresponds with the time required by the host to lay down a wound periderm and to produce a wound-gum barrier (54), both of which seem adequate to delimit the fungus. After the cessation of the activity of the fungus the history of the lesions was identical with that of the checks, and in many cases (Series 16-24, 35-43, Figs. 2 and 3) considerable decrease in size occurred before the onset of the first dormant season. From October to April, when the host is inactive, *S. fructicola* seems to be able to spread through the host tissue at a rate limited mainly by the prevailing temperature. This accounts for the large amount of necrosis in Series 4 and 5 in the fall of 1931 (see Fig. 2 and Plate II, Figs. 5 and 6) when temperatures were relatively high throughout November. In the fall of 1932 (Series 27 and 28, Fig. 3) low temperatures after leaf fall evidently restricted infection by the fungus (Plate III, Fig. 5).

While temperature may sometimes be the limiting factor, it is not the only one which determines the rate of spread of *S. fructicola*. There is some evidence that humidity plays an important part in this connection. For example, in Series 17 (Fig. 2), the relative humidity was comparatively high (mean 82%) for the first day or so after inoculation, but the mean temperature was below 40° F. and the sky overcast so that branch temperature would approximate air temperature (51). Then when the temperature rose to the point where the fungus could grow, the humidity dropped (mean 59%). Later, when neither factor was limiting, periderm formation and wound-gum production had advanced sufficiently to delay the progress of the pathogen. Likewise in Series 19 (Fig. 2), while temperatures were favorable, humidity was low (mean 57%) and necrosis was slight. In Series 18, 20 and 21 (Fig. 2), however, where necrosis was severe, the mean relative humidity for the first ten days in each case was 72–73% and the temperature well above 45° F., except for the first three days in Series 18. In Series 16 (Fig. 2), although the mean temperature was for the most part adversely low, there was a period of about 18 hours during the second day when both temperature and humidity were favorable and the fungus evidently was able to gain entry. The absolute figures for relative humidity as given above are not in themselves especially significant, but they serve to indicate periods of drought or of dampness, confirmed by the continuous records of humidity. In the spring months, the succulent nature of the actively growing tissues would seem to be conducive to the growth of the fungus, once infection had occurred. In the height of

the summer, when tissues were firmer and branch temperatures higher and probably above the optimum for *S. fructicola*, especially on sunny days, there was less necrosis. This unfavorable set of conditions was in all probability supplemented also by the extreme diurnal fluctuations in humidity characteristic of summer weather. In any case in series inoculated during the summer months of 1932 and 1933, the least necrosis was coincidental with the highest mean temperature for the critical 10-day period following inoculation (Series 23 and 43, Figs. 2 and 3). In Series 44 the amount of damage was also small, but here a rainfall of one and one-half inches during the first 12 hours may conceivably have washed off much of the inoculum. Again, during the winter of 1932–33 the open wounds increased in size to a much less extent than the slits. It seems reasonable to suppose that this difference in behavior was due in large measure to a greater tendency on the part of the larger wounds to dry out.

The percentage of active cankers appearing after inoculation with *S. fructicola* (4.6%) was somewhat lower than in the case of *V. leucostoma* (6.8%) and slightly greater than in the case of checks (2%). These differences are scarcely significant except for the suggestion that delay in healing and the presence of necrotic tissue may provide more opportunity for natural infection with canker-producing organisms. There is no doubt that the active cankers which developed both in the series involving *S. fructicola* and in the checks were due to contamination, as valsoid pycnidia were almost invariably present. Here also re-isolations yielded *Valsa cincta*. Although in Series 45 (Fig. 1) *S. fructicola* was credited with 100% active cankers in the spring of 1934, this was undoubtedly due to the late date at which infection took place in 1933. However, according to the criteria the expectation is that the future history of this series will not be different from that of earlier ones.

There is no disputing the fact that some cankers on the peach, in their incipient stages, may be caused by infection with *S. fructicola*, but these experiments clearly demonstrate the invalidity of the term "brown rot cankers" as applied to peach cankers, especially if they are perennial. In nature, *S. fructicola* gains access to the twig, if not solely at least chiefly, through blighted blossoms and rotting fruit, but since the majority of cankers are found to originate at other points (55), then *S. fructicola* can be regarded as responsible for only a relatively small number of cankers. Undoubtedly the lesions initiated by *S. fructicola* are as subject to infection with canker-producing organisms, for example *Valsa cincta*, as any other sort of wound, but unless such infection occurs they are not likely to become typical peach cankers.

Checks

In considering the check wounds, two or three points of interest may be noted. In general, the growth of callus seems to be somewhat slower in wounds made early in the dormant season than in those made in spring. The exposed tissues of the former tend to dry out during the winter months as the slight enlargement of such wounds indicates, while in the latter the

healing processes become operative very soon after wounding. In most instances, also, the rate of closure was greater during the first growing season after wounding, during which time the wounds on the larger, more vigorously growing branches became almost, if not completely, healed. As a rule, the growth of callus was least in the direction parallel to the long axis of the branch and greatest at right angles, that is to say, as the wounds were overgrown by the callus developing along their sides rather than at their ends, decrease in length was achieved largely by the meeting of the lateral calli at the ends of the wounds. Consequently, large decreases during the second growing season (as in *V. cincta* 22, 23, 40, 42, 43; *S. fructicola* 27-29, 36, 42-44, and checks 20, 22 and 24, Figs. 2 and 3) do not denote rapid healing at that time so much as the meeting of lateral calli in wounds which were almost closed and therefore long and very narrow at the end of the first growing season. The callus development in wounds on smaller branches varied with the growth rate of the branch, so that in some cases, where the growth of the branch was negligible, little change occurred in the size of the wound. Such cases became apparent as a horizontal tendency in the curves, in the portion covering the third growing season (Series 1–24, Figs. 2 and 3).

To conclude, convincing evidence has been obtained that, in so far as peach canker is concerned, the fungus herein designated as *Valsa cincta* is in itself quite capable of acting as a primary causal organism infecting fresh wounds, as well as a successor to lesion-forming pathogens such as *Sclerotinia fructicola*. In either case, it is the important factor in the production of perennial cankers. *V. leucostoma*, on the other hand, is relatively unimportant as a primary parasite, whatever role it may assume under other circumstances in the later stages of the disease. However, it should be emphasized that, while there is no doubt of the major significance of *V. cincta* in the Niagara Peninsula of Ontario, no claim is made that this organism is the only pathogen concerned in the formation of perennial cankers on the peach.

Acknowledgment

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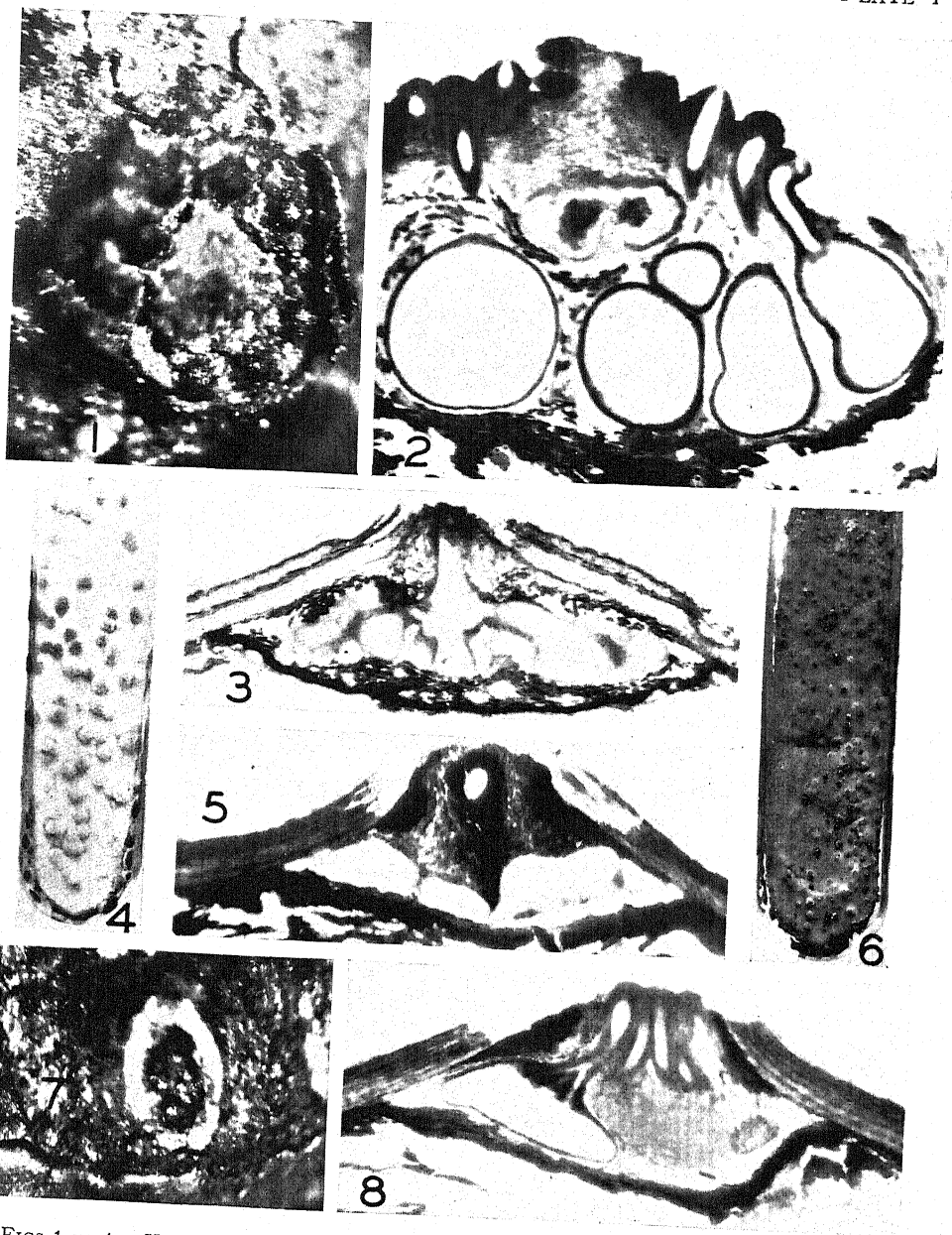
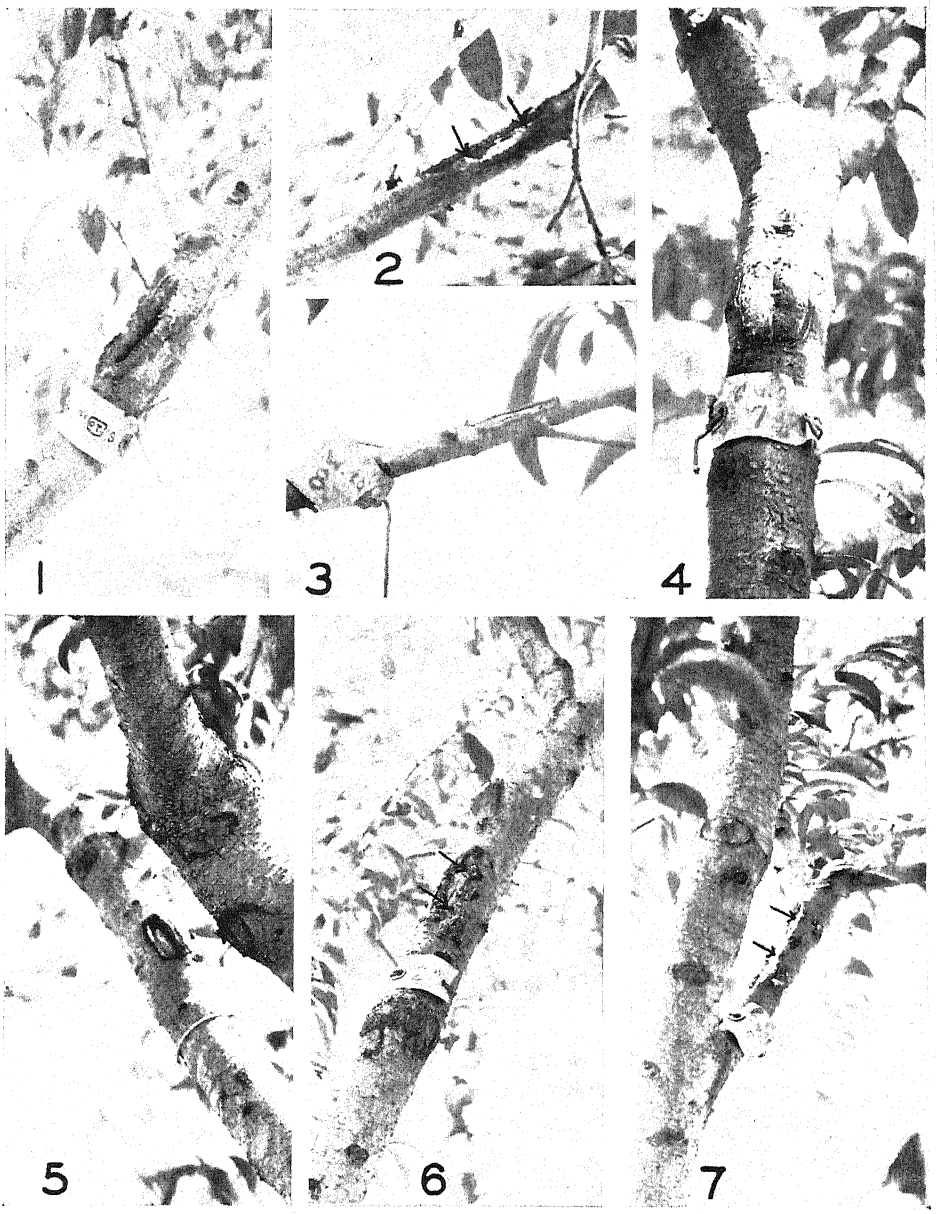


FIGS 1 TO 4. *Valsa cincta.* FIGS. 5 TO 8. *Valsa leucostoma.* FIGS. 1 AND 7. *Erumpent discs of perithecial stromata.* FIGS. 2 AND 8. *Median sections through perithecial stromata.* FIGS. 3 AND 5. *Median sections through pycnidial stromata.* FIGS. 4 AND 6. *Cultures on potato-dextrose agar. Magnification of photomicrographs approximately 40 diameters. Cultures natural size.*

Typical wounds from series 5, made and inoculated October 19, 1931; photographed June 19, 1933. FIGS. 1 AND 2. *With Valsa cincta, three badly cankered lesions.* FIGS. 3 AND 4. *With V. leucostoma, one healed and two still open but not cankered.* FIGS. 5 AND 6. *With S. fructicola. Note extent of original necrosis, followed by healing.* FIGS. 7 AND 8. *Checks, one healed and one open wound. Arrows, where present, indicate original size of wound.*



Typical wounds, photographed June 19, 1933, comparing effects of inoculation in growing and in dormant seasons. FIGS. 1 AND 2. *S. fructicola, Series 20, inoculated May 30, 1932.* FIGS. 3 AND 4. *V. cincta, Series 23, inoculated July 11, 1932.* FIG. 5. *S. fructicola, Series 33, inoculated Dec. 3, 1932.* FIGS. 6 AND 7. *V. cincta, Series 33, also inoculated Dec. 3, 1932. Note: necrosis occurred in 1, 2, 6 and 7 but not in 3, 4 and 5. Arrows where present, indicate original size of wound.*

FLAX STUDIES

I. THE RELATION BETWEEN WEIGHT PER MEASURED BUSHEL, WEIGHT PER THOUSAND KERNELS AND OIL CONTENT OF FLAXSEED[1]

By W. F. Geddes[2] and F. H. Lehberg[3]

Abstract

Determinations of test weight per bushel, weight per 1000 kernels and oil content made on 146 samples of Western Canadian flaxseed, 119 of which graded No. 1 C.W., 16 No. 2 C.W. and 11 No. 3 C.W., revealed that while the grades were differentiated in regard to test weight per bushel and weight per 1000 kernels, the mean oil contents for the three grades were not significantly different. Test weight per bushel is of little significance as an index of oil content. While the degree of association of weight per thousand kernels with oil content is somewhat closer, it is not sufficiently high to permit satisfactory prediction of this variable.

Introduction

In the inspection and grading of the cereal grains, the primary object is to classify the product into groups of differing quality in regard to its suitability for the particular purpose which it is tended to serve. Since chemical determinations are in most instances impractical owing to the time, the cost, and the volume of work required, it is necessary in practical grain grading to utilize certain physical characteristics which can be readily determined or estimated. In the grading of flaxseed in Canada and the United States, test weight per bushel, content of damaged seeds, presence of foreign material, and moisture content are the principal factors employed. Since linseed oil is the most valuable product obtained from flaxseed, the grading factors should reflect the quantity and quality of oil in the seed. In 1927 Coleman and Fellows (1) reported the results of a study of the relation between oil content of flaxseed and such variables as test weight per bushel, size of seed, moisture content, damaged and foreign seed content, and found that the oil content was not closely related to the percentage of certain types of damaged kernels nor to test weight per bushel. These workers reported, as a result of an examination of some 2000 samples of flaxseed, that test weight per bushel is of little significance as an index of oil content, the mean oil contents for samples weighing from 47 to 53 lb. per bushel being practically equal, although there appeared to be a tendency for samples of high test weight to contain less oil than those of lower weight.

Preliminary experiments undertaken in this laboratory indicated that the weight per 1000 kernels is more closely related to the oil content of flaxseed than test weight per bushel, which led to the study of the relation between weight per thousand kernels, test weight per bushel and oil content of flaxseed reported in this paper.

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Experimental

The experimental material consisted of 146 samples of Western Canadian flaxseed taken from cars sampled in the Winnipeg Inspection Division, during the past two crop years. The production of flaxseed in Western Canada has fallen to such a low level that it was found difficult to secure many samples, particularly in the grades lower than No. 1 C.W. Of the total of 146 samples collected, 119 graded No. 1 C.W., 16 No. 2 C.W. and 11 No. 3 C.W. However, these represented approximately 40% of the combined Western Canadian crop inspected at Winnipeg for the two crop years, and hence may be regarded as fairly representative.

Foreign material was removed from the samples by means of a Perkins flax cleaner, and the cleaned seed employed for the analytical studies. Test weight per bushel was determined in duplicate by means of an Imperial pint or ½ pint bucket, a Cox funnel being used, and the seed weighed with a weight per bushel balance. As the samples had been stored in the laboratory for several weeks, their moisture contents were practically identical (approximately 5.0%). In any event, Coleman and Fellows (1), have shown that, as compared with other cereals, variations in the moisture content of flaxseed have little influence on test weight per bushel.

TABLE I

STATISTICAL CONSTANTS

Grade No.	No. of samples	Means			Standard deviations			Coefficients of variability		
		Wt. per bushel, (x) lb.	Wt. per 1000 kernels, (y) gm.	Oil cont. (z) %	Wt. per bushel, (x) lb.	Wt. per 1000 kernels, (y) gm.	Oil cont. (z) %	Wt. per bushel, (x) %	Wt. per 1000 kernels, (y) %	Oil cont. (z) %
1 C.W.	119	54.52	5.287	40.76	1.29	0.950	1.52	2.4	18.0	3.7
2 C.W.	16	50.04	4.817	41.47	2.23	0.436	1.52	4.4	8.8	3.7
3 C.W.	11	48.50	4.714	40.65	1.78	0.674	2.14	3.7	14.3	5.3
All grades	146	53.58	5.192	40.83	2.48	0.912	1.58	4.6	17.6	3.9
Variance between grades (F)		14.00	3.77	1.51						

Variance within grades at 5% pt. F. = 3.06

Simple correlation coefficients

Correlation		1 C.W.	2 C.W.	3 C.W.	All grades
Wt. per bu. (x) × oil content (z)	r_{xz}	−.225	.001	.326	−.126
Wt. per 1000 kernels (y) × oil content (z)	r_{yz}	.487	.327	.672	.444
Wt. per bu. (x) × wt. per 1000 kernels (y)	r_{xy}	−.157	−.135	.040	.106
At 5% pt. r =		.182	.497	.602	.164

In determining weight per 1000 kernels, 500 kernels were counted out at random, in duplicate, weighed to ± 0.01 gm. and the results computed to a dry matter basis. Oil content was determined in duplicate by the ethyl ether extraction method outlined in the second paper of this series (2).

The statistical constants computed on the means of the duplicate values for weight per bushel, weight per 1000 kernels and oil content, recorded in Table I, summarize the results obtained. From the mean values of these variables and the "F" values for the ratios of the variances contributed by the differences between grades to the corresponding variances between the samples within the grades, it will be noted that, while there is a significant decrease in test weight per bushel and weight per 1000 kernels with a decrease in grade, the mean values for oil content are not significantly different. The simple correlations reveal that in grade No. 1 C.W. there is a slight tendency for high oil content to be associated with low test weight per bushel, although such a relation does not exist for the other two grades. On the other hand, weight per 1000 kernels is positively correlated with oil content, and the correlations are of a higher order of magnitude than those involving test weight per bushel. However, the degree of association is not sufficiently high to permit a satisfactory prediction of oil content by its use; moreover, the determination is laborious and for this reason alone, weight per 1000 kernels is unsuitable as a grading factor.

The results on this limited series of samples of Canadian flaxseed fully confirm the observations of Coleman and Fellows (1) that weight per bushel is of little significance as an index of oil content, and indicate the necessity of developing a convenient and rapid method for estimating oil content if the grading of flax is to be placed on a scientific basis.

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FLAX STUDIES

II. AN IMPROVED REFRACTOMETRIC METHOD FOR ESTIMATING THE OIL CONTENT OF FLAXSEED[1]

By W. F. Geddes[2] and F. H. Lehberg[3]

Abstract

The method proposed by Coleman and Fellows, based upon the change in refractive index of halowax, an impure substituted monochlornaphthalene, on dilution with linseed oil extracted from the ground sample, is improved by drying the sample before extraction, grinding in a Hobart mill, filtration for 15 minutes, and the use of a special refractometer with illumination by a 40 watt light. The use of 4 cc. of solvent and extraction at 70° C. was confirmed. The correlation between halowax extract scale reading and oil content as determined by ethyl ether extraction was 0.95; the standard error of prediction of oil content by the refractometric method was 0.59%. The refractometer readings were not appreciably affected by variations in the refractive index of linseed oil as determined on the ether extract. The correlation between the refractive index and the iodine value of the ether extract was 0.70 with a standard error of prediction of 3.1 units.

Further improvement is obtained by the use of a solvent consisting of approximately 50% by volume of halowax and α-bromnaphthalene which gives a standard error of prediction of 0.39%. By slight changes in the proportions, the refractive index of this solvent may be adjusted to a constant value, permitting the use of a permanent conversion chart. Addition of anhydrous sodium sulphate during the extraction obviates the necessity of preliminary drying. With the modifications indicated, the method is suitable for routine determinations on large numbers of samples.

Introduction

In the first paper of this series, Geddes and Lehberg (4) reported that neither test weight per bushel nor weight per thousand kernels was of any practical utility as a measure of the oil content of Canadian flaxseed. The conventional chemical methods for determining the oil content of flaxseed, involving extraction with solvents, as pointed out in the previous paper, are not applicable for routine grading purposes and the experiments reported here were accordingly undertaken to investigate the possibility of a rapid optical method.

The use of an optical method for the measurement of the oil content of oil-bearing seeds was first proposed in 1920 by Wesson (5), who developed a refractometric method for cottonseed meal and cottonseed meats. This method is based upon the change in the refractive index of a solvent known commercially as halowax, an impure substituted monochlornaphthalene, as it became diluted with cottonseed oil extracted from the sample. This solvent is well suited to a test of this nature, since it is essential, in order to secure the greatest accuracy, that the indices of refraction of the vegetable oil and the solvent should differ widely. Halowax oil has a refractive index

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of 1.635 at 25° C., which is considerably higher than the refractive indices of the vegetable oils. Other fundamental requirements of a suitable solvent which are possessed by halowax are that it should be non-volatile, non-inflammable, inexpensive, and possess a low coefficient of expansion.

Working with cottonseed meal, a material of low oil content, Wesson found his method gave values in good agreement with those obtained by the usual ether extraction method but in the cottonseed meats, which are of higher oil content, the agreement was not so satisfactory. Coleman and Fellows (1, 2) accordingly made a study of the method and introduced certain modifications in technique to render the test applicable to the estimation of the oil content of flaxseed and other oil-bearing seeds. Their technique for flaxseed consisted of triturating 2 gm. of the finely ground sample in a 3-in. porcelain mortar previously heated to 70° C. with 4 cc. halowax and 1 to 2 gm. of fine sea sand for at least 2 min., filtering and determining the refractive index of the filtrate at 25° C. with an Abbé refractometer, an instrument which could be read to the fifth place. The refractive index found was converted to oil content by means of a conversion table prepared by adding definite amounts of a petroleum ether extract of flaxseed to a known quantity of halowax oil and noting the refractive indices of the solutions. The relation between the change in the refractive index of halowax oil and the linseed oil content of the solution was found to be linear, each 1% of linseed oil present reducing the refractive index of the solvent by 0.00191 units. The method was applied to the analysis of 120 samples of flaxseed and the results compared with those obtained by the A.O.A.C. petroleum ether extraction method. The greatest individual variation found was 0.33%, while the oil content of 80.8% of the samples tested agreed within $\pm 0.2\%$, and 45.8% within $\pm 0.1\%$ by the two methods. The average deviation between the results by the two methods has been computed by the present authors and found to be 0.12%, while the standard deviation of the differences was 0.08%.

In preliminary tests by this laboratory, the application of the refractometric method, as described by Coleman and Fellows (2), to the estimation of the oil content of Western Canadian flaxseed, did not give results in satisfactory agreement with the ether extraction method. In these studies, the samples were weighed out on a dry matter basis and the presence of varying amounts of moisture was found to introduce serious errors due to cloudy filtrates, which made reading difficult, and probably also to interference with the efficiency of extraction of the linseed oil by the solvent. Considerable improvement was obtained by removal of moisture before trituration with halowax, thus eliminating the formation of emulsions, but it seemed essential that a refractometer capable of being read accurately to the fifth decimal place be employed in order to bring the accuracy of the method within desirable limits. Moreover, the method employed by Coleman and Fellows in preparing the conversion table is based on the assumption that the linseed oil extracted by halowax corresponds in refractive index to that obtained with petroleum ether. While it is presumed that the petroleum ether extract used

was a composite from several samples, thus taking into consideration variations in the refractive indices of linseed oil from different samples, it would seem preferable to determine the oil content and refractive index of a halowax extract of a series of samples and prepare a conversion table on the basis of the regression of oil content on refractive index.

In view of these considerations, the study reported here was undertaken with the object of developing a more satisfactory refractometric method for estimating the oil content of Canadian flaxseed.

Experimental

For the purpose of this study a special refractometer, constructed by the Zeiss Company according to specifications supplied by the laboratory, was obtained. This instrument, shown in Fig. 1, combines the dipping tube and eye piece with the Abbé water-jacketed head. Three prisms are provided, the measuring range of each covering individually the refractive index limits of linseed oil, halowax, and the linseed-oil–solvent mixtures as follows:

Scale Reading	*Refractive Index*
−5.0 to +105.0	1.46514 to 1.48986
−5.0 to +105.0	1.62191 to 1.65303
−5.0 to +105.0	1.62438 to 1.58908

By thus limiting the range of each prism head, a greater degree of accuracy is obtainable; the accuracy with which the arbitrary scale values of the instrument may be read corresponds to a determination of refractive index to the fifth decimal place, and by interpolation to the sixth place.

Preparatory to the analysis of a series of samples of flaxseed, it seemed advisable first to study the influence of such variables as fineness of grind, flaxseed-solvent ratio, temperature and time of extraction, and light source, on the results by the refractometric method.

In regard to light source, daylight did not give a sufficiently sharp line of demarcation to permit accurate readings being made, while sodium light and that given by a 40 watt, inside frosted, incandescent, electric light bulb were equally satisfactory; for practical reasons the latter was employed.

Tests conducted with samples ground to different degress of fineness showed, as pointed out by Coleman and Fellows (1, 2), that uniformity in grinding is essential for consistent results. These workers recommend grinding in an experimental flour mill, fitted with 6 × 6 in. rolls, corrugated 40 to the inch and operated at a differential of $1\frac{1}{2}$ to 1. In our hands, this method did not give a uniformly ground product, the pulp tending to remain in the corrugations and to accumulate on the housing beneath the front roll, rendering cleaning of the mill between samples tedious and difficult. As a result of pressure, the pulp which accumulated in the corrugations is very oily and the portions of this which become detached do not mix readily with the more floury portions of the pulp. The grind that could be obtained in the most uniform manner without any appreciable day-to-day variation was a fine pulp grind obtained

on the Hobart Model 6 burr mill (equipped with stationary burr No. 4317, No. 2 R.N. Sta. MCH No. 6 Ex PG and rotary burr No. 4318 No. 2 P.H. Rot. MCH No. 6 Ex PG), with a setting of from 5.0 to 5.5.

Owing to the nature of the material, a measure of the fineness of grind, by means of sieving, cannot be obtained without first extracting the oil. The results of typical sieving tests on the residues after extraction with ethyl ether are shown in Table I.

TABLE I

TYPICAL SIEVING TEST ON ETHER-EXTRACTED FLAXSEED PULP

Flour silk No.	Meshes per square inch	Weight retained
0 XX	1444	6 – 10
2 XX	2916	30 – 33
4 XX	3844	5 – 6
Total		41 – 49%
Total passing through No. 4 XX =		51 – 59%

In the Coleman and Fellows method, 2 gm. of ground flaxseed is extracted with 4 cc. of halowax at 70° C. It seemed desirable, in studying the effect of variations in oil-solvent ratio, to conduct the extractions at room temperature as well as at 70° C. since, if satisfactory results could be obtained at the lower temperature, the test would be considerably simplified. Five samples of flaxseed, ranging in oil content from approximately 38% to 43% were extracted at 24° and 70° C. respectively, using 3, 4, 5 and 6 cc. halowax and 2 gm. moisture-free flax pulp. Quadruplicate extractions were made for each temperature and solvent-oil ratio and quadruplicate readings on each extract. A statistical study of the resulting data indicated that extraction at 70° C. gave a much better differentiation between samples than extraction at 20° C. Also 4 cc. solvent gave the greatest range between samples with the minimum variability within replicates, the results therefore confirming the conditions selected by Coleman and Fellows in regard to these particulars.

While variations in maceration time of from 1 to 4 min. were found to be without any significant influence on the refractive index readings, investigation showed that the first few drops of the filtrate were not representative of the extract, the best results being obtained with a filtration time of approximately 15 min., sufficient time also being allowed for the filtrate to acquire the temperature of the prism, *i.e.*, 25° C., before taking readings.

An essential requirement for accuracy and rapidity in conducting refractometer readings is the provision of a convenient and reliable constant temperature control. Early tests were made using the hanging wall-tank and gas-heated coil apparatus, but this method was abandoned owing to its lack of efficiency. The present equipment, as shown in Fig. 1, consists of a constant pressure tower fitted with a valve to regulate the flow of water through a water-cooled rheostat. By proper adjustment of the valve and the sliding contact of the rheostat the effluent can be easily held within $\pm 0.1°$ C., the variations normally being within $\pm 0.05°$ C.

On the basis of these preliminary experiments, the following method was employed for the purpose of studying the relation between oil content and refractive index of the halowax extract.

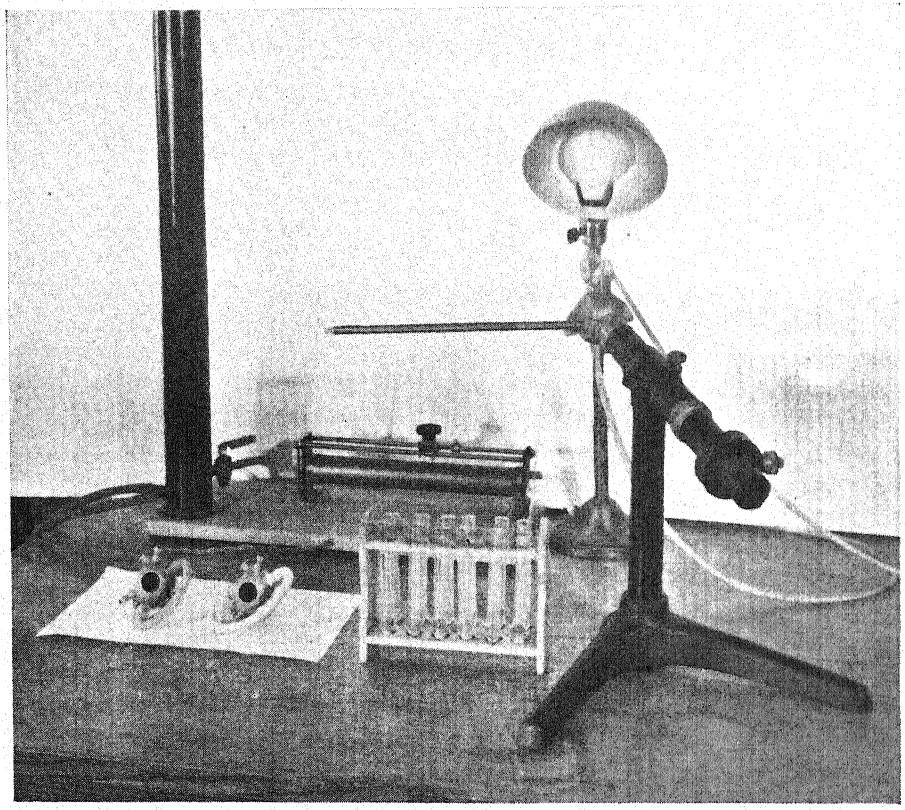

FIG. 1. *Refractometer and temperature control device.*

The flaxseed is ground to a fine pulp in the Hobart burr mill and then dried overnight in the vacuum oven at 98°–100° C.

Two grams of the dried material is accurately weighed and triturated for at least 1 min. in a mortar previously heated to 70° C. with 1 gm. of fine sand and 4 cc. halowax.

The mixture is filtered through a 70 mm. folded Prat-Dumas filter paper and the filtrate collected in a test tube.

After filtration for approximately 15 min. the refractive index of the filtrate is determined at 25° C., one minute being allowed for the extract to acquire the temperature of the prisms before taking the readings.

Triplicate halowax extractions with duplicate scale readings on each extraction were made and the mean values employed for the subsequent statistical studies reported here.

In regard to the determination of the oil content of flaxseed, there is apparently no standard method in general use, as indicated in the replies received to a questionnaire submitted to research and industrial laboratories early in 1934. Not only was there considerable variation in the type of extraction apparatus employed but the times of extraction and the solvents used differed

widely. For this reason it seems essential to describe in some detail the method employed in this laboratory. A 10 gm. sample of the flax pulp, ground in the Hobart burr mill as described for the refractometric studies, is d ied overnight *in vacuo* at 98°–100° C. and extracted on an electrically heated water bath for 16 hr. with anhydrous alcohol-free and peroxide-free ethyl ether in a Soxhlet extractor, using Whatman double thickness extraction thimbles, and a siphoning rate of 1 per minute. The ether extract is filtered through a sintered glass filter and transferred directly to a tared 125 cc. Erlenmeyer flask by suction, the extraction flask being washed three times with fresh solvent. This procedure is necessary to remove traces of starch which are inevitably present in the extract. The excess ether is distilled off on a water bath maintained at approximately 70° C., the extract dried *in vacuo* for 3 hr. at 98°–100° C. at a pressure not exceeding 25 mm. mercury, and the oil content computed to a dry matter basis.

It has been found that increasing the time of extraction beyond 16 hr. does not increase the quantity of oil recovered. However, if the extracted sample is transferred to a mortar and re-ground with fine carborundum or sand and re-extracted with ethyl ether for a further 12 hr., a slight additional quantity of oil is obtained. The re-grinding increases the tendency for starch to be present in the extract and it is particularly necessary that the extract be filtered before evaporation. The quantity of oil recovered from a number of re-ground, previously extracted samples varied from 0.12 to 0.30% with an average of 0.16%. In view of the relative constancy of the additional oil and the inconvenience of re-extraction, single extractions only were made in the present study.

In order to determine whether variations in the refractive index of the ether extract had any appreciable influence on the corresponding values obtained on the halowax extract, refractometric scale readings were made on the ether extracts at 25° C. The iodine values of these extracts were also determined in duplicate according to Wijs' method.

Sixty-eight samples of flaxseed grown in different districts of Western Canada in the crop years of 1931, 1932 and 1933 and for the most part grading No. 1 C.W., were submitted to the analytical determinations outlined above and the statistical constants computed from the respective mean values obtained for the individual samples are given in Table II.

The close relation between the scale reading of halowax extracts and oil content, as determined by ether extraction, is shown by the high and significant correlation $r_{yz} = .948$. As $1 - r^2$ may be regarded as an estimate of the fraction of the total variance of halowax scale reading independent of oil content, 90% of the variance is attributable to the correlation. That the scale readings on halowax extracts are not appreciably influenced by variations in the refractive index of linseed oil, as determined on ethyl ether extracts, is shown by the partial correlation $r_{ys \cdot x} = .122$. This result is quite surprising and suggests that either the solvent actions of ethyl ether and halowax are dissimilar or that the refractive index of linseed oil is modified in an

TABLE II

STATISTICAL CONSTANTS FOR DATA ON OIL CONTENT, SCALE READING OF HALOWAX EXTRACT, IODINE VALUE AND SCALE READING OF ETHER EXTRACT

(N = 68)

Means, standard deviations and ranges

		Mean	S.D.	Range
Oil content, %	(x)	40.37	1.75	37.0 – 43.8
Halowax extract scale reading	(y)	45.63	3.62	38.5 – 51.7
Iodine value (Wijs)	(z)	184.38	4.36	174.6 – 196.5
Ether extract scale reading	(s)	40.55	4.43	33.4 – 64.0

Simple correlation coefficients

		r	t*
Oil content × halowax extract scale reading	r_{xy}	.948	24.0
Oil content × iodine value	r_{xz}	−.258	2.1
Oil content × ether extract scale reading	r_{xs}	.246	2.0
Halowax extract scale reading × iodine value	r_{yz}	−.241	2.0
Iodine value × ether extract scale reading	r_{zs}	−.700	8.0
Halowax extract scale reading × ether extract scale reading	r_{ys}	.271	2.3

*At 5% point t = 1.9
t = 1.0

First order partial correlation coefficients $r_{ys \cdot x}$ = .122

Regression coefficients

		B	t
Oil content on halowax extract scale reading	b_{xy}	.458	24.0
Iodine value on ether extract scale reading	b_{zs}	−.689	8.0

Linear regression equations

	Standard error of prediction
Estimation of oil content (x) from halowax scale reading (y) $x = 19.471 + .458y$	0.59%
Estimation of iodine value (z) from ether extract scale reading (s) $z = 212.38 - .689s$	3.12

entirely irregular manner by the laboratory manipulations involved in the ether extract determination. This point is now being investigated by comparing the refractive indices and iodine values of ether extracts of flaxseed with the corresponding values determined on linseed oil expressed by means of a Carver laboratory press.

Since the scale readings of halowax extracts depend to such a large extent on the oil content of flaxseed, they may be employed as an estimate of oil content by having recourse to the regression equation $x = 19.471 + .458\,y$,

which gives the most probable value of x for determined values of y. The linearity of regression was tested by the analysis of variance procedure, (Fisher (3)) and the deviations from the regression line were not significant. Hence, the standard error of a single predicted observation is defined by $\sqrt{1 - r^2}\,\sigma x$, which in this instance equals 0.59%.

For routine work the estimation of iodine value from the refractive index of linseed oil would be of considerable utility. Accordingly, the regression of iodine value on scale reading of the ether extract was computed and is given in Table I. The relation between these variables was found to be linear, the standard error of a single predicted iodine value being ± 3.1.

It is of interest to note that there is a slight tendency for low iodine values to be associated with high oil content, as shown by the correlation $r_{xz} = -.258$.

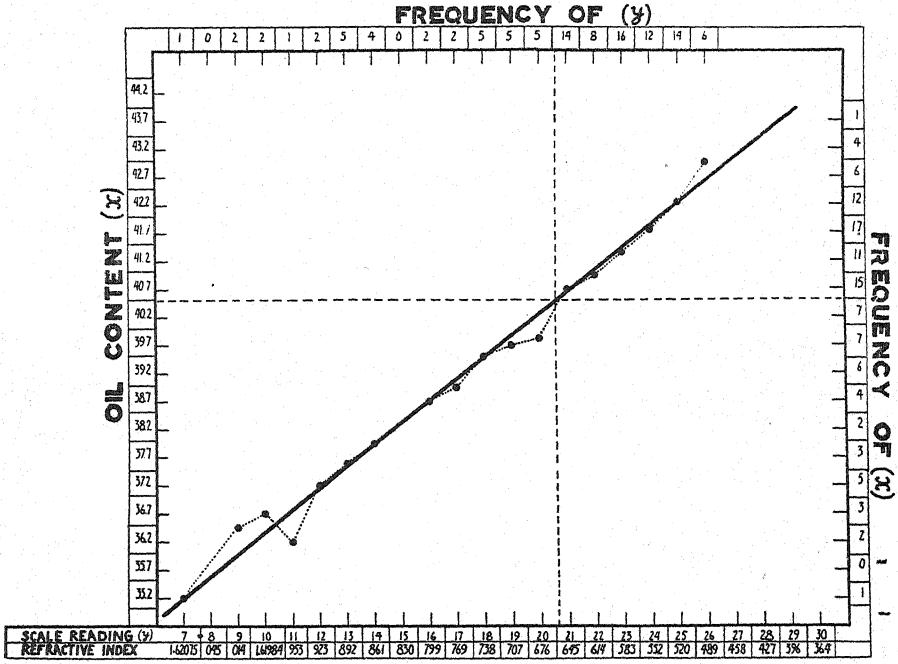

FIG. 2. *Regression of oil content of flaxseed on refractive index and scale reading of halowax-α-bromnaphthalene (50 : 50 by volume) extracts.*

The accuracy of prediction of the oil content of flaxseed by the refractometric method, using halowax as solvent, leaves much to be desired. Moreover, as this substance is impure, its refractive index is not constant throughout various shipments. This necessitates frequent revision of the conversion table. It was therefore decided to investigate the possibility of other available linseed oil solvents of satisfactory physical properties. Three solvents were considered and their characteristics and approximate costs as compared with halowax, are given in Table III.

TABLE III

Solvent	Refractive index, at 25° C.	Boiling point, °C.	Specific gravity	Approximate cost per 500 gm. $
Monochlornaphthalene (halowax)	1.635	246	1.368	0.75
α-methylnaphthalene	1.618	243	1.001	1.50
Tetra bromethane	1.638	151	2.92	3.00
α-bromnaphthalene	1.6558	281	1.487	3.00

The refractive index of α-methylnaphthalene is not sufficiently high to warrant consideration; also the practical grade is colored and the refined too high in cost. While tetrabromethane has a refractive index similar to halowax, the high specific gravity would render its cost excessive for routine testing. On the other hand, α-bromnaphthalene has a higher refractive index than that of monochlornaphthalene, thus making it a superior solvent since the greater the difference in refractive indices of the solvent and linseed oil, the greater the attainable accuracy.

Since the refractive index of bromnaphthalene fell beyond the measuring range of any of the prisms at hand, and also in view of the relatively high cost of this solvent, it was decided to investigate the utility of a mixture of approximately 50% by volume with halowax. The mixed solvent possesses the advantage that its refractive index can be adjusted to a constant value (1.64461 at 25° C.) by varying slightly the percentages of its components, thus eliminating the inconvenience of adjusting the conversion table for each new shipment, as is required for halowax, and making it possible to prepare a conversion table for permanent use.

Refractometric determinations were made on 106 samples of flaxseed, using as the solvent a mixture of approximately 50% by volume of α-bromnaphthalene and α-chlornaphthalene (halowax), and the method previously outlined for the halowax extraction. As before, the oil content was determined by ethyl ether extraction and the iodine number and refractive index of the ether extract determined. The data obtained are summarized by the statistical constants recorded in Table IV.

The correlation between oil content of flaxseed and scale reading of the mixed solvent extracts, $r_{xy} = .977$, is significantly higher than the corresponding correlation of .948 for the halowax data. As in the instance of the halowax extractions, the regression of oil content on scale reading was found to be linear. The regression coefficient $b_{xy} = 0.393$ is significantly lower than that for halowax ($b_{xy} = 0.458$) so that a unit change in scale reading corresponds to a smaller increment of change in oil content for the new solvent, thus increasing the accuracy of estimation, as shown by the lower standard error of prediction of $\pm 0.39\%$. The regression line is shown in Fig. 2 with the frequencies for each scale reading in 1-unit class intervals and the actual means and frequencies for oil content in 0.5% class intervals. It

TABLE IV

STATISTICAL CONSTANTS FOR DATA ON OIL CONTENT, SCALE READING OF HALOWAX-BROMNAPHTHALENE EXTRACT, IODINE VALUE AND SCALE READING OF ETHER EXTRACT

(N = 106)

Means, standard deviations and ranges

		Mean	S.D.	Range
Oil content, %	(x)	40.48	1.84	35.1 – 43.3
Halowax-bromnaphthalene extract scale reading	(y)	20.58	4.57	6.5 – 26.2
Iodine value (Wijs)	(z)	183.79	3.61	179.0 – 193.3
Ether extract scale reading	(s)	41.47	3.57	33.5 – 50.3

Simple correlation coefficients

		r	t*
Oil content × halowax-bromnaphthalene extract scale reading	r_{xy}	.977	46.7
Oil content × iodine value	r_{xz}	−.160	1.6
Halowax-bromnaphthalene extract scale reading × iodine value	r_{yz}	−.129	1.3
Halowax-bromnaphthalene extract scale reading × ether extract scale reading	r_{ys}	.094	1.0
Iodine value × ether extract scale reading	r_{zs}	−.647	8.7

*At 5% pt. t = 1.9

Regression coefficients

		B	t
Oil content on halowax-bromnaphthalene extract scale reading	b_{xy}	.393	46.7
Iodine value on ether extract scale reading	b_{zs}	−.646	8.7

Linear regression equations

	Standard error of prediction
Estimation of oil content from halowax-bromnaphthalene extract scale reading $x = 32.40 + .393\,y$	0.39%
Estimation of iodine value from ether extract scale reading $z = 210.59 - .646\,s$	2.75

will be noted that the mean values for oil content fall very close to the regression line with the exception of two classes for which the frequencies are low. The refractometric scale readings were not converted to refractive indices in making the statistical computations, as in routine practice it would be much more convenient to convert the instrumental readings directly to oil content. However, the refractive indices corresponding to unit scale values are given in the regression graph and also in the tentative conversion chart reproduced in Table V, which was computed from the regression equation $x = 32.40 + .393\,y$.

In regard to the regression of the iodine value of ethyl ether extracts on the refractometric scale readings of the extract an analysis of variance showed that the deviations from regression were not significant. The regression graph is given in Fig. 3 and it will be noted from Table IV that the standard error of prediction of iodine value (Wijs) from the regression equation $z = 210.59 - .646s$ is ± 2.75. In this equation and the conversion chart computed therefrom no cognizance has been taken of the residual oil which may be recovered by re-grinding and re-extraction, since this procedure is not in general use.

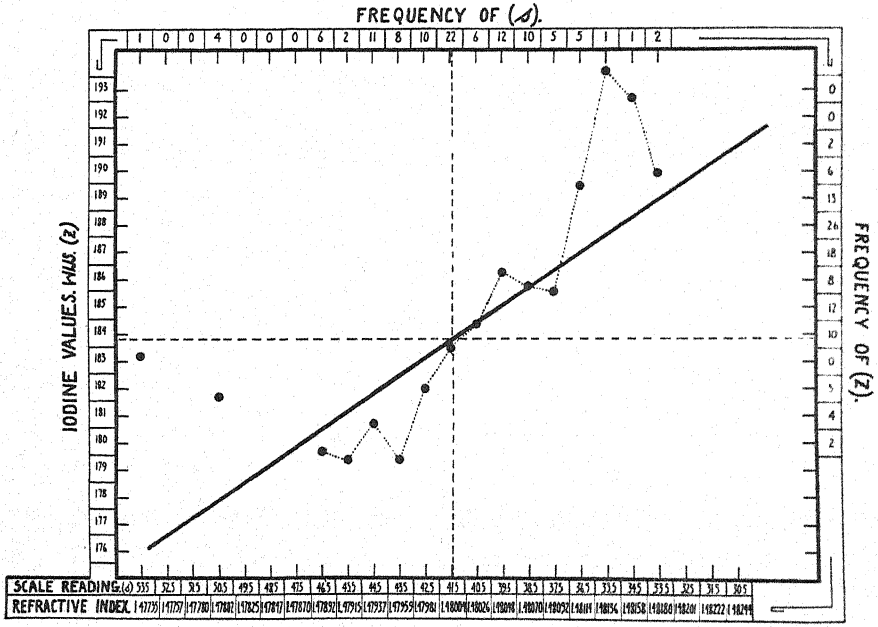

FIG. 3. *Regression of iodine value on refractive index and scale reading of ethyl ether extracts of flaxseed.*

Casual observations indicate that the refractive index of ethyl ether extract of flaxseed is quite sensitive to variations in the temperature at which the ether is distilled off upon completion of extraction and that the changes in refractive index do not appear to be accompanied by corresponding changes in iodine value. While every attempt has been made to standardize the technique in this series, it seems quite possible that a greater degree of accuracy in predicting iodine value from the refractive index of linseed oil would be obtainable in the instance of expressed oils; also the variations noted in the refractive index of ethyl ether extracts of individual samples may, as previously indicated, be responsible for the insignificant influence of the refractive index of the oil upon that of the halogenated naphthalene extracts.

In view of the fact that the halowax and halowax-bromnaphthalene extractions were not conducted on precisely the same samples throughout, and of

TABLE V

TENTATIVE CONVERSION CHART FOR COMPUTING OIL CONTENTS FROM SCALE READING OR REFRACTIVE INDEX OF HALOWAX–α-BROMNAPHTHALENE EXTRACT OF FLAXSEED

Scale reading, (25° C.)	n. (25° C.)	Oil content, %	Scale reading, (25° C.)	n. (25° C.)	Oil content, %	Scale reading, (25° C.)	n. (25° C.)	Oil content, %
7.0	1.62075	35.2	14.2	1.61855	38.0	21.4	1.61633	40.8
7.2	069	35.2	14.4	849	38.0	21.6	626	40.9
7.4	063	35.3	14.6	842	38.1	21.8	620	41.0
7.6	057	35.4	14.8	836	38.2	22.0	614	41.0
7.8	051	35.5	15.0	830	38.3	22.2	608	41.1
8.0	045	35.5	15.2	824	38.4	22.4	602	41.2
8.2	039	35.6	15.4	818	38.4	22.6	595	41.3
8.4	033	35.7	15.6	811	38.5	22.8	589	41.4
8.6	026	35.8	15.8	805	38.6	23.0	583	41.4
8.8	020	35.8	16.0	799	38.7	23.2	577	41.5
9.0	014	35.9	16.2	793	38.8	23.4	571	41.6
9.2	008	36.0	16.4	787	38.8	23.6	564	41.7
9.4	002	36.1	16.6	781	38.9	23.8	558	41.8
9.6	1.61996	36.2	16.8	775	39.0	24.0	552	41.8
9.8	990	36.2	17.0	769	39.1	24.2	546	41.9
10.0	984	36.3	17.2	763	39.2	24.4	539	42.0
10.2	978	36.4	17.4	757	39.2	24.6	533	42.1
10.4	972	36.5	17.6	750	39.3	24.8	526	42.1
10.6	965	36.6	17.8	744	39.4	25.0	520	42.2
10.8	959	36.6	18.0	738	39.5	25.2	514	42.3
11.0	953	36.7	18.2	732	39.6	25.4	508	42.4
11.2	947	36.8	18.4	726	39.6	25.6	501	42.4
11.4	941	36.9	18.6	719	39.7	25.8	495	42.5
11.6	935	37.0	18.8	713	39.8	26.0	489	42.6
11.8	929	37.0	19.0	707	39.9	26.2	483	42.7
12.0	923	37.1	19.2	701	39.9	26.4	477	42.8
12.2	917	37.2	19.4	695	40.0	26.6	470	42.8
12.4	911	37.3	19.6	688	40.1	26.8	464	42.9
12.6	904	37.4	19.8	682	40.2	27.0	458	43.0
12.8	898	37.4	20.0	676	40.2	27.2	452	43.1
13.0	892	37.5	20.2	670	40.3	27.4	446	43.2
13.2	886	37.6	20.4	664	40.4	27.6	439	43.2
13.4	880	37.7	20.6	657	40.5	27.8	433	43.3
13.6	873	37.7	20.8	651	40.6	28.0	427	43.4
13.8	867	37.8	21.0	645	40.6			
14.0	861	37.9	21.2	639	40.7			

Refractive index of halowax–α-bromnaphthalene mixture = 1·64461 at 25° C.

the more satisfactory results secured by the use of the latter solvent, a further series of 100 samples was extracted in duplicate, employing three solvents, namely: halowax, a mixture of approximately 50% by volume of halowax and α-bromnaphthalene, and a 20–80 mixture by volume of halowax and bromnaphthalene.

The relations between oil content and the refractometric readings obtained for the first two solvents agreed closely with those previously reported in detail. However, the degree of association between the means of duplicate scale readings and oil content for the 20–80 halowax-bromnaphthalene extraction was of a lower order of magnitude than for either of the solvents previously employed, and the differences between duplicates were significantly higher.

In view of the excellent agreement obtained between the two series of determinations employing the 50–50 mixture, and also taking into consideration its lower cost, no further investigation was made of the 20–80 mixture to determine the reasons for the apparently anomalous results obtained by its use. It should be mentioned, however, that it was necessary to employ two sets of prisms in order to cover the range in refractive index encountered and this may possibly account in part for the differences found, although the instrument was carefully re-standardized upon changing the prisms.

It will be recalled that, owing to the difficulty with cloudy filtrates when samples contain more than approximately 5% moisture, all samples were dried prior to extraction. This procedure detracts from the rapidity of the test and its value for routine purposes. Experiments were accordingly undertaken to determine whether satisfactory results could be secured by the optical method when anhydrous salts were employed for the removal of moisture. Three anhydrous salts, sodium sulphate, copper sulphate and sodium carbonate, in slight excess, were tried with satisfactory results, the first mentioned appearing to yield slightly clearer filtrates and lower variability between replicates.

TABLE VI

TYPICAL RESULTS OBTAINED BY IMPROVED REFRACTOMETRIC METHOD ON SAMPLES DRIED IN THE VACUUM OVEN *vs.* WITH ANHYDROUS SODIUM SULPHATE

Sample No.	Estimated oil content (50% mixture halowax bromnaphthalene extraction)	
	Dried *in vacuo*, %	Dried with Na_2SO_4, %
1	40.3	40.3
2	40.8	40.9
3	40.2	40.2
4	41.4	41.4
5	40.6	40.6
6	41.6	41.8
7	42.1	42.1
8	42.2	42.2
9	43.4	43.2
10	43.0	43.1
11	41.3	41.4
12	41.1	41.1

A number of samples were tested at their original moisture content, 0.15 gm. Na_2SO_4* being added to the pulp prior to the addition of the 50–50 halowax-bromnaphthalene solvent, and the results compared with those obtained on corresponding samples dried in the vacuum oven prior to extraction. A portion of the data is presented in Table VI, from which it will be noted that excellent agreement was obtained.

Discussion

The results of this study indicate that a mixture of approximately 50% by volume of α-bromnaphthalene and halowax, with a refractive index of 1.64461 at 25° C., offers distinct advantages over halowax as a solvent for linseed oil in the estimation of the oil content of flaxseed by means of the

* *Subsequent to the completion of these experiments, Leithe (Leithe, Wolfgang, Angew, Chem. 47 : 734-736, 1934) has published the details of a refractometric method for the determination of the oil content of oil bearing seeds in which the sample is pulverized with sea sand and anhydrous sodium sulphate prior to extraction with a gasoline fraction (b.p. 90°–100° C.).*

refractometer. The mixed solvent has a higher refractive index than halowax alone and by slight variations in the composition of the mixture the refractive index of the solvent may be adjusted to a constant value, thus rendering it possible to prepare a conversion table for permanent use. The accuracy of estimation of oil content, employing the procedure outlined, may be regarded as fairly satisfactory, especially when the simplicity and rapidity of the method are taken into consideration and also considering the possibility that variations in refractive index of the oil may exert a significant influence on the refractometric readings. While the present study has failed to show such an influence, it will be recalled that the refractive indices were determined on ether-extracted oils and there is some indication that such determinations may be unreliable as a measure of the true refractive index of the linseed oil as extracted by the halogenated naphthalene solvents. Under these circumstances, the conversion chart given in Table V may be regarded as tentative. If the studies now in progress indicate that variations in the refractive index of cold-pressed oil significantly influence the refractometric readings, a correction can be readily applied.

The use of anhydrous sodium sulphate for removal of moisture permits determinations to be made on samples as received without oven drying. The cost of the solvent required for triplicate determinations is approximately $0.063 per sample and the test can be performed with only a limited amount of preliminary training. It would therefore appear that the method would prove of considerable practical utility for routine determinations on large numbers of samples.

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VARIABILITY IN EXPERIMENTAL BAKING

II. THE INFLUENCE OF MECHANICAL MOULDING IN REDUCING THE VARIABILITY IN LOAF VOLUME BETWEEN LABORATORIES[1]

BY W. F. GEDDES,[2] R. K. LARMOUR[3] AND J. G. MALLOCH[4]

Abstract

The results of replicate bakings of five flours by the simple and bromate formulas in three laboratories, using both hand and machine moulding, showed that differences in the manual manipulation of doughs during moulding by experienced operators are relatively unimportant in causing variability between replicates. Mechanical moulding slightly reduced the variability between laboratories but the mean volumes for the three laboratories fell in the same order for hand and machine moulding, indicating that certain systematic factors were operating which affected the hand and machine results similarly. Machine moulding slightly reduced the differences in mean loaf volume obtained by three bakers of varying experience working in the same laboratory, while in the instance of three experienced bakers, hand moulding gave the lower variability between bakers.

Introduction

In the first paper (1) of this series, the results of an extensive series of experiments, carried out with the Thomson laboratory model loaf moulder in the Cereal Chemical laboratory of the University of Manitoba in 1930, were presented. The experiments were designed to determine the value of mechanical moulding as compared with hand moulding in reducing (i), the variability between replicate bakings of the same flour by one operator, (ii) the variation within the same day and on different days, (iii) the variability between operators. With the most satisfactory adjustment of the mechanical moulder, mechanical moulding gave only a slightly lower variability in the loaf volume of replicate bakings than hand moulding, but the machine-moulded loaves were smaller in volume, coarser in texture and duller in crumb color. Machine moulding did not reduce the difference between days nor the trends in loaf volume within days. In fact, when the days were arranged in order of increasing mean loaf volume the same order was obtained by hand and machine moulding, indicating the operation of some systematic factors that influenced hand and machine results similarly. Experiments conducted by

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bakers of different degrees of experience gave results which indicated that inexperience in moulding is not an important factor contributing to variability between replicates. However, the personal element in both punching and moulding was found to contribute to the variability between bakers, individuality in moulding being somewhat the more important factor.

A study of the factors contributing to variability in experimental baking is of particular interest to the Associate Committee on Grain Research, National Research Council of Canada, since the experimental work in connection with the various projects of the Committee is replicated in the different laboratories co-operating with the Committee. While the standardization of the baking methods employed has resulted in fair relative agreement between the collaborating institutions, wide differences in the absolute values for any given flour are obtained.

Although individuality both in punching and moulding technique was found to contribute to the variability between operators in the Manitoba laboratory, the relatively greater importance of moulding technique indicated that the introduction of mechanical moulding might effect a significant reduction in the variability between laboratories. The studies reported in this paper were accordingly undertaken to secure information on this point.

Experimental

Upon completion of the tests in the Manitoba laboratory, the mechanical moulder was forwarded to the University of Saskatchewan, together with 50-lb. samples of the five flours that had been used by the former laboratory in the second series of experiments. A description of the flours is given in

TABLE I

DESCRIPTION OF FLOURS

Flour No.	Description	Bleaching treatment	Chemical analysis, 13.5% moisture basis	
			Protein, %	Ash, %
5	Commercially milled, 50% patent	Alsop (nitrogen peroxide) + ½ oz. Novadel (benzoyl peroxide) per bbl.	12.3	0.39
6	Commercially milled, bottom 60%	Unbleached	14.0	0.60
7	Commercially milled, 96% patent	1.5 gm. Agene (nitrogen trichloride) per bbl. + Alsop	13.2	0.49
8	Commercially milled, 2nd clear (bottom 15%)	3 gm. Agene per bbl.	15.4	1.05
9	Experimentally milled, straight grade	None	13.9	—

Table I. After preliminary tests, these flours were baked by an experienced baker (A) by the simple and bromate formulas, using the same flour absorptions as the Manitoba laboratory. Fifty loaves of one flour were baked daily, the first 25 being hand moulded and the second 25 machine moulded, the adjustment of the moulder being that specified by the Manitoba laboratory, namely, the sheeting rolls set at "2" on the dial and the depth of the compression chamber at the upper end adjusted to 1.28 in. corresponding to a depth of 1.603 in. at the exit end, the latter measurement being made vertically to the drum.

In order to secure further information on the utility of mechanical moulding in reducing the variability between operators, flour Number 7 was also baked by two other operators, using the bromate formula, each baking 25 loaves by hand moulding and 25 by machine moulding. The moulder was then shipped to the University of Alberta where a similar series of experiments was carried out with samples of the same flours, thus providing comparative data for hand and machine moulding in the three laboratories. As 50 loaves of each flour were baked by each formula and moulding procedure in the Manitoba laboratory, 25 were selected at random in order to simplify the statistical calculations required in comparing the data of the three laboratories. The number of loaves baked for each flour, formula, moulding method, and laboratory, together with the corresponding mean loaf volumes, are summarized in Table II. Additional statistical constants for Laboratories B and C are given in Table III; corresponding data for Laboratory A are given in Table XLI of the paper by Geddes *et al.* (1) which should be consulted for details regarding the statistical methods employed.

In all series, with the exception of flours Nos. 7 and 9 baked by the bromate formula in Laboratory C, machine moulding gave lower average loaf volumes than hand moulding, but the differences are not by any means constant. However, the mean responses to bromate of corresponding flours are in general of the same order of magnitude for the two methods of moulding.

In Laboratory A, machine moulding was found to effect a slight but significant reduction in the variability of replicate bakings. In Laboratory B, two flours, Nos. 7 and 9, baked by the bromate method, show significant differences in variability, one exhibiting greater and the other less variation by hand than by machine moulding. In Laboratory C, flours Nos. 6 and 9 baked by the simple formula are the only ones in which the differences in variability are significant and in both of these machine moulding had the advantage. The variabilities for all flours combined are not a satisfactory basis of comparison since these are influenced to a large extent by the variations in the general loaf volume levels of the different series of replicates included.

In order to determine whether machine moulding reduces the variability between replicates when the data for all flours and both baking formulas are considered and also to bring out other information, particularly the utility of mechanical moulding in reducing the differences between labor-

TABLE II

MEAN LOAF VOLUMES AND NUMBER OF LOAVES BAKED

Laboratory	Formula	No. 5		No. 6		No. 7		No. 8		No. 9		All flours	
		No.	Mean, cc.	No.	Mean, cc.	No.	Mean, cc.	No.	Mean, cc.	No.	Mean, cc.	No.	Mean, cc.
Hand moulding													
A	Simple	25	628.0	25	555.4	25	637.4	25	601.3	25	570.2	125	598.5
	Bromate	25	681.3	25	782.9	25	766.4	25	699.2	25	646.9	125	715.4
	Simple + bromate	50	654.6	50	669.2	50	701.9	50	650.3	50	608.6	250	656.9
B	Simple	25	496.8	25	551.4	25	610.8	25	539.8	25	513.6	125	562.5
	Bromate	25	648.0	24	731.9	25	739.8	25	696.0	25	624.6	124	687.7
	Simple + bromate	50	622.4	49	639.8	50	675.3	50	617.9	50	569.1	249	624.8
C	Simple	25	513.1	25	493.0	25	519.7	25	513.7	25	499.1	125	507.7
	Bromate	25	562.0	25	649.5	25	634.5	25	586.3	25	570.5	125	600.6
	Simple + bromate	50	537.5	50	571.2	50	577.1	50	550.0	50	534.8	250	554.1
A + B + C	Simple	75	579.3	75	533.3	75	589.3	75	551.6	75	527.6	375	556.2
	Bromate	75	630.4	74	721.3	75	713.6	75	660.5	75	614.0	374	667.8
	Simple + bromate	150	604.9	149	626.6	150	651.4	150	606.1	150	570.8	749	612.0
Machine moulding													
A	Simple	25	595.4	25	522.5	25	591.3	25	566.6	25	529.8	125	561.1
	Bromate	25	634.1	25	741.0	25	714.6	25	661.8	25	597.0	125	669.7
	Simple + bromate	50	614.7	50	631.8	50	652.9	50	614.2	50	563.4	250	615.4
B	Simple	25	574.8	25	532.4	25	561.4	25	516.2	25	484.0	125	533.8
	Bromate	25	621.0	24	683.5	25	701.2	25	669.4	25	592.6	124	653.3
	Simple + bromate	50	597.9	49	606.4	50	631.3	50	592.8	50	538.3	249	593.3
C	Simple	25	510.4	25	474.0	25	499.4	25	458.4	25	476.0	125	483.6
	Bromate	25	552.3	25	624.5	25	650.3	25	563.2	25	575.6	125	593.2
	Simple + bromate	50	531.4	50	549.2	50	574.8	50	510.8	50	525.8	250	538.4
A + B + C	Simple	75	560.2	75	509.6	75	550.7	75	513.7	75	496.6	375	526.2
	Bromate	75	602.5	74	681.9	75	688.7	75	631.4	75	588.4	374	638.7
	Simple + bromate	150	581.3	149	595.7	150	619.7	150	572.6	150	542.5	749	582.4

TABLE III

MACHINE *versus* HAND MOULDING BY EXPERIENCED OPERATORS IN DIFFERENT LABORATORIES

(Statistical constants pertaining to loaf volume data)

Flour No.	Baking formula	No. of loaves	Mean loaf volume				Diff. hand − machine, cc.	Diff. in L.V. as % L.V. by hand	Range in loaf volume		Standard deviation in L.V.		Sign of diff. in variability $Z/\sigma z$	Coefficient of variability in L.V.	
			Hand moulded, cc.	Machine moulded, cc.	Response to bromate				Hand moulded, cc.	Machine moulded, cc.	Hand moulded, cc.	Machine moulded, cc.		Hand moulded, %	Machine moulded, %
					Hand moulded, cc.	Machine moulded, cc.									
							Laboratory B								
5	Simple	25	596.8	574.8	51.2	46.2	22.0	3.69	50	60	13.03	13.38	0.13	2.18	2.33
5	Bromate	25	648.0	621.0			27.0	4.17	80	55	17.38	15.23	0.64	2.68	2.45
6	Simple	25	551.4	532.4	180.5	151.1	19.0	3.45	20	20	5.39	6.02	0.55	0.98	1.13
6	Bromate	24	731.9	683.5			48.4	6.61	40	60	12.06	16.68	1.56	1.65	2.44
7	Simple	25	610.8	561.4	129.0	139.8	49.4	8.09	50	40	11.72	10.73	0.43	1.92	1.91
7	Bromate	25	739.8	701.2			38.6	5.22	75	80	14.32	21.51	1.99	1.94	3.07
8	Simple	25	539.8	516.2	156.2	153.2	23.6	4.37	40	45	11.70	12.98	0.51	2.17	2.52
8	Bromate	25	696.0	669.4			26.6	3.82	70	40	16.00	7.79	3.53	2.30	1.16
9	Simple	25	513.6	484.0	111.0	108.6	29.6	5.76	80	70	26.40	18.38	1.77	5.14	3.80
9	Bromate	25	624.6	592.6			32.0	5.12	80	60	17.37	14.70	0.82	2.78	2.48
Entire series	Simple	125	562.5	533.8	125.2	119.5	28.7	5.10	155	150	39.28	34.85	1.33	6.98	6.53
	Bromate	124	687.7	653.3			34.4	5.00	190	190	47.98	43.53	1.08	6.98	6.66
	Simple + bromate	249	624.8	593.3			31.5	5.04	300	300	76.42	71.60	1.02	12.23	12.07
							Laboratory C								
5	Simple	25	513.1	510.4	48.9	41.9	2.7	0.53	100	63	21.08	16.96	1.07	4.11	3.32
5	Bromate	25	562.0	552.3			9.7	1.73	58	56	16.18	17.51	0.38	2.88	3.17
6	Simple	25	493.0	474.0	156.5	150.5	19.0	3.85	58	41	12.68	8.49	1.96	2.57	1.79
6	Bromate	25	649.5	624.5			25.0	3.85	131	144	28.84	34.13	0.82	4.44	5.46
7	Simple	25	519.7	499.4	114.8	150.9	20.3	3.91	57	50	15.26	12.07	1.15	2.94	2.42
7	Bromate	25	634.5	650.3			−15.8	2.49	90	94	24.61	26.43	0.35	3.88	4.06
8	Simple	25	513.7	458.4	72.6	104.8	55.3	10.76	106	84	19.32	20.88	0.38	3.76	4.56
8	Bromate	25	586.3	563.2			23.1	3.94	50	65	13.73	14.29	0.20	2.34	2.54
9	Simple	25	499.1	476.0	71.4	99.6	23.1	4.63	89	54	21.45	11.72	2.96	4.30	2.46
9	Bromate	25	570.5	575.6			− 5.1	8.94	85	101	19.19	27.12	1.69	3.36	4.71
Entire series	Simple	125	507.7	483.6	92.9	109.6	24.1	4.75	128	123	20.84	23.81	1.48	4.10	4.92
	Bromate	125	600.6	593.2			7.4	1.23	214	178	40.99	45.24	1.10	6.82	7.63
	Simple + bromate	250	554.1	538.4			15.7	2.83	286	283	56.68	65.64	2.31	10.23	12.19

atories, the analyses of variance summarized in Table IV were made. The standard errors given at the foot of the table were computed from the corresponding error variances (differences between replicates) given in the variance analysis tables. The standard errors of single determinations are in reality pooled standard deviations for the differences between replicates and they have been compared by means of the Z test. While the random errors for machine moulding are significantly lower than for hand moulding in the instance of Laboratory A, there is no significant difference for Laboratories B and C or for all laboratories combined. The random errors for the different laboratories by each moulding procedure may also be compared by the Z test. Laboratory C has a significantly higher error for both hand and machine moulding than Laboratory B, and is also significantly higher than Laboratory A in the instance of machine moulding. It must, therefore, be concluded that the higher experimental error for this laboratory is due to factors other than moulding technique.

The Z values for "differences between flours" measure the significance of the differences between the mean loaf volumes (simple and bromate formulas combined) of the various flours. The corresponding Z values for hand and machine moulding are very similar, indicating that there is no essential difference between the two moulding procedures in their utility in differentiating between flours. For example, from Table II it will be noted that, by both procedures and in all laboratories, Flour 9 gave the lowest mean volume and Flour 7 the highest. The ranges of the means for flours for Laboratory A are 93.3 cc. and 89.5 cc. for hand and machine moulding respectively, for Laboratory B 106.2 and 93.0 cc., for Laboratory C 42.3 and 49.0 cc. and for all laboratories combined, 80.6 cc. and 77.2 cc.

The Z values for the "differences between formulas" are, in general, also of the same order of magnitude for hand and machine moulding. In Laboratory A the range between the simple and bromate formula is 116.9 cc. and 108.6 cc. for hand and machine moulding respectively, for Laboratory B 125.3 and 119.5 cc., for Laboratory C 93.1 and 109.6 cc., and for the three laboratories combined 111.6 and 112.5 cc.

Similarly, the Z values for interaction "flours $\times$ baking formulas" correspond quite closely for the two methods of moulding, and it may be concluded that from the standpoints of differentiating between flours, measuring the response to bromate and the differential behavior of flours to this oxidizing agent, machine moulding gives essentially the same information as hand moulding.

The Z values for the differences between laboratories are of particular interest. For hand moulding $Z = 3.7126$ and for machine moulding 3.4587, thus indicating that machine moulding tends to reduce slightly the variability between laboratories. Thus, from Table II it will be noted that the mean loaf volumes for Laboratories A, B and C are 657, 625 and 554 cc. respectively, for hand moulding, while the corresponding values for machine moulding are 615, 593 and 538 cc. The ranges in the mean volumes for the three laboratories are 103 cc. for hand and 77 cc. for machine moulding.

TABLE IV

MACHINE VERSUS HAND MOULDING BY EXPERIENCED OPERATORS

(Analysis of variance for loaf volume data)

Variance due to	Degrees of freedom	Variance		Z		5% point
		Hand moulding	Machine moulding	Hand moulding	Machine moulding	
Laboratory A						
Differences between flours	4	57,063.6	54,759.6	2.5451	2.8007	0.4397
Differences between baking formulas	1	854,042.1	736,905.4	3.8980	4.0764	0.6780
Interaction, flours × formulas	4	57,457.4	59,653.1	2.5485	2.8194	0.4397
Differences between replicates	240	351.3	212.2			
Laboratory B						
Differences between flours	4	74,080.6	58,241.5	2.8489	2.7961	0.4397
Differences between baking formulas	1	976,092.4	889,622.4	4.1381	4.1592	0.6780
Interaction, flours × formulas	4	30,628.5	25,470.3	2.4072	2.3825	0.4397
Differences between replicates	239	248.4	217.1			
Laboratory C						
Differences between flours	4	18,578.8	30,219.6	1.9079	2.1190	0.4397
Differences between baking formulas	1	538,982.6	750,431.2	3.5918	3.7250	0.6780
Interaction, flours × formulas	4	22,945.6	25,286.3	2.0135	2.0298	0.4397
Differences between replicates	240	409.1	436.3			
Laboratories A, B and C Combined						
Differences between flours	4	133,173.8	122,156.2	2.8892	2.8744	0.4345
Differences between laboratories	2	691,278.8	392,974.4	3.7126	3.4587	0.5507
Differences between baking formulas	1	2,332,427.1	2,371,136.0	4.3207	4.3573	0.6746
Interactions,						
Flours × laboratories	8	8,274.6	10,532.3	1.4999	1.6490	0.3341
Flours × baking formulas	4	96,606.2	91,722.6	2.7287	2.7312	0.4345
Laboratories × baking formulas	2	18,345.0	2,911.5	1.8981	1.0061	0.5507
Differences between replicates	727	412.0	389.2			

Standard Errors

Laboratory	Hand, cc.	Machine, cc.	Significance of difference, Hand vs. machine	
			Z	5% point
A	18.74	14.57	0.2522	0.1064
B	15.76	14.73	0.0675	0.1064
C	20.23	20.89	0.0322	0.1064
A, B and C	20.30	19.73	0.0284	0.0610

The variation among the three laboratories is very wide, and since machine moulding only reduced the difference by approximately one-third, the lower loaf volumes of Laboratory C must be due largely to factors other than moulding technique.

The significant interaction "flours × laboratories" is of importance since it implies that the different laboratories did not obtain the same relative results with the various flours. This interaction is based on the mean loaf volumes of each flour for both baking formulas and moulding procedures. These are recorded in Table V, the flours being arranged in order of increasing loaf volume as determined in Laboratory A. The order in which the flours are placed is almost identical for the three laboratories, the interaction being chiefly due to the similarity in the mean volumes of flours Nos. 9, 8 and 5 in Laboratory C.

TABLE V

INTERACTION TABLE FOR "FLOURS × LABORATORIES" MEAN LOAF VOLUMES

(Combined results for simple and bromate formulas and for hand and machine moulding)

Flour No.	Laboratory			
	A, cc.	B, cc.	C, cc.	A+B+C, cc.
9	586	554	530	557
8	632	605	530	589
5	635	610	534	593
6	650	623	560	611
7	677	653	576	636
All flours	636	609	546	597

A statistical summary of the data obtained by the different bakers in Laboratories B and C is given in Table VI. In Laboratory B all the bakers were trained technicians, whereas in Laboratory C, bakers A and B were both experienced but the latter only baked at irregular intervals; baker C was the experimental miller and had no previous baking experience whatever.

Considering first the results obtained in Laboratory B, bakers A and B secured higher variability between replicates for machine moulding although the differences are not significant. The pooled standard deviation for the three technicians, calculated from the error variance, is 16.8 cc. and 19.8 cc. for hand and machine moulding respectively. Since the Z value for the difference is 0.1640 with a 5% point of 0.1933, the higher variability for machine moulding is not statistically significant. The range in mean loaf volume for the three bakers is 7.4 cc. and 18.8 cc. for hand and machine moulding respectively. Since the corresponding Z values for the "differences between bakers" are 0.1592 and 0.8858 with 5% points of 0.5696, the differences in mean loaf volume obtained by the three bakers are significant for machine but not for hand moulding.

The results in Laboratory B appear to warrant the conclusion that experienced bakers trained in one laboratory are likely to produce more consistent results by hand than by machine moulding. The use of a mechanical moulder, would, however, be justified, if through its use more consistent results could

TABLE VI

HAND VERSUS MACHINE MOULDING BY DIFFERENT BAKERS

(Statistical constants for loaf volume data)

		Baker			All bakers
		A	B	C	
Laboratory B—Flour No. 7 baked by bromate formula					
Number of replicates,	Hand	25	25	25	75
	Machine	25	25	25	75
Mean, cc.,	Hand	739.8	747.2	741.2	742.7
	Machine	701.2	707.4	688.6	699.1
Mean difference (Hand − machine), cc.		38.6	39.8	52.6	43.6
Standard deviation (σ) cc.,	Hand	14.61	16.65	18.78	16.85
	Machine	21.95	20.87	15.91	21.02
Significance of difference in S.D., Z/σ_Z		1.41	0.78	0.57	1.90
Coefficient of variability (C.V.), %,	Hand	1.98	2.23	2.53	2.27
	Machine	3.13	2.95	2.31	3.01

Analyses of variance

Variance due to	Degrees of freedom	Variance		Z		5% point
		Hand	Machine	Hand	Machine	
Difference between bakers	2	386.50	2,294.50	0.1592	0.8858	0.5696
Difference between replicates	72	281.13	390.22			
Standard error of 1 determination		16.77	19.75			
Standard error of means for bakers		3.35	3.95			

		Baker			All bakers
		A	B	C	
Laboratory C—Flour No. 5 baked by bromate formula					
Number of replicates	Hand	25	24	25	74
	Machine	25	24	25	74
Mean, cc.,	Hand	562.0	591.6	515.5	555.9
	Machine	552.3	597.4	547.9	565.4
Mean difference (Hand − machine), cc.		9.7	−5.8	−32.4	−9.5
Standard deviation (σ) cc.,	Hand	16.52	19.98	18.59	36.33
	Machine	17.87	21.57	15.43	28.90
Significance of difference in S.D., Z/σ_Z		0.27	0.27	0.65	2.00
Coefficient of variability (C.V.), %,	Hand	2.94	3.38	3.61	6.54
	Machine	3.24	3.61	2.82	5.11

TABLE VI—*Concluded*

HAND *versus* MACHINE MOULDING BY DIFFERENT BAKERS—*Concluded*

(Statistical constants for loaf volume data)

Variance due to	Degrees of freedom	Variance		*Z*		5% point
		Hand	Machine	Hand	Machine	
		Analyses of variance				
Difference between bakers	2	36,162.24	18,226.70	2.3359	1.9920	0.5699
Differences between replicates	71	338.34	339.20			
Standard error of 1 determination		18.39	18.42			
Standard error of means for bakers		3.68	3.68			

be secured by inexperienced technicians. The data obtained in Laboratory C are of interest in this connection since baker C had no baking experience whatever and baker B was out of practice. The two experienced bakers secured slightly lower variability between replicates by hand moulding while machine moulding gave the lowest variability for baker C. In no instance, however, are the differences significant and the pooled error variances for hand and machine moulding are practically identical. It is interesting to note that in hand moulding, the relative variability (coefficient of variation) for the three bakers tends to vary inversely as their baking experience, a trend which is in line with previous observations in this connection.

Considering the mean loaf volumes, bakers B and C secured higher volumes by machine than by hand moulding, with the result that the spread between the bakers is reduced from 86 cc. for hand moulding to 46.5 cc. for machine moulding. However the variance for the "differences between bakers" is still highly significant and factors in addition to moulding technique must therefore materially contribute to the low mean loaf volumes obtained by the inexperienced operator. Geddes *et al.* (1) have previously shown that inexperienced operators tend to secure lower loaf volumes and higher relative variabilities than experienced bakers and that the manual operations involved in both punching and moulding contribute to the differences between bakers.

Discussion

These studies show quite clearly that differences in the manual manipulation of doughs during moulding are relatively unimportant in relation to the total effect of other factors causing variability between replicates. While mechanical moulding reduced, to some extent, the variability between experienced bakers working in different laboratories, the differences were still very great and the bakers fell in the same order in regard to mean loaf volume for both hand and machine moulding, indicating that some systematic factors were operating which affected similarly both the hand and machine

results. That factors other than moulding contribute greatly to the variability between bakers is also indicated by the similar order of the mean loaf volumes for hand and machine moulding obtained by the three experienced bakers in Laboratory B; in this instance the variability between operators was greater for machine moulding. The data secured in Laboratory C by operators of varying experience indicate that mechanical moulding enables an inexperienced baker to secure mean volumes more closely approaching those obtained by trained technicians.

The present study supplements and, in general, confirms the results reported by Geddes *et al.* (1) who found that the personal element, both in punching and moulding, contributed to the variability between bakers and, accordingly, stated that mechanical moulding alone could not be expected to eliminate the large differences in mean loaf volume which different operators secure in replicate bakings of the same flour.

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CAMBIAL ACTIVITY IN POPLAR WITH PARTICULAR REFERENCE TO POLARITY PHENOMENA[1]

By A. B. Brown[2]

Abstract

Ringing experiments with the aspen poplar, *Populus tremuloides* Michx., lead to the conclusion that cambial activity is definitely not rigidly or unconditionally polar in its development in the root. A much greater development of cambial activity in the morphologically upward direction was obtained in these experiments than has hitherto been observed. It is suggested that the concept of polarity, applied to cambial activity as a process, must be defined in terms of a *tendency* to develop in the morphologically downward direction, rather than in the morphologically upward direction, in roots and stems. Polarity in relation to cambial activity in general is discussed briefly.

Introduction

In a previous communication, the writer (1) showed that cambial activity, emanating from sucker shoots of poplar, flows apparently almost entirely in the distal direction on entering the parent root, resulting in marked thickening of the root on the distal side, except in cases where sucker shoots occur very close to one another, when this distal thickening may not be obvious. This type of behavior is expressed invariably however, in the grain of the wood, which runs longitudinally down the sucker shoot and continues likewise along the root in the distal or acropetal direction. In the crotch, *i.e.*, the region common to both shoot and root, the grain runs longitudinally at all points on the distal side, but on the proximal side it divides, turns sharply through an angle of 90°, and then swings round to run longitudinally and distally. As a result, in longitudinal radial sections of the root and sucker shoot at the crotch, the xylem elements are cut longitudinally on the distal side, whereas on the proximal side, in the region common to both root and shoot, the xylem elements are cut more or less transversely. It was also shown that the tissue orientations just described are anticipated at a very early stage; *viz.*, around the base of the sucker bud from which the sucker shoot ultimately develops. Vascular connection between the sucker bud and the root xylem is attained by the development of a peg of tracheids. As a result of the interaction between the normal acropetal flow of cambial activity in the root and this vascular peg, the tissues subsequently formed turn sharply on the proximal side and orient themselves around the peg, as seen in longitudinal tangential sections, in a form essentially similar to that of a flow-pattern. In longitudinal radial section, the root xylem is cut transversely just proximal to the vascular peg and longitudinally on the distal side.

In the early part of the summer, soon after the basipetal flow of cambial activity from the shoot has reached the root, a well defined gradient of cambial activity, estimated in terms of xylem formation, can be detected, running from the base of the shoot, along the root in the distal direction. At the same time however, a feeble gradient in the opposite direction can be observed,

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running from the base of the shoot, along the root in the proximal direction. Certain investigators, who will be referred to later, postulate that cambial activity is polar, in so far as it can only travel in the morphologically downward direction in the stem, and in the acropetal direction in the root. However, in view of the gradient of xylem formation in the *proximal* direction, feeble though it may be, it would appear that polarity of cambial activity is not complete and unconditional. It was thought worth while therefore, to investigate further the polarity of cambial development in this material, and the following experiments were designed expressly to yield some information on this subject. The fact that cambial activity is evidently not completely polar in development suggested to the writer (1) the possibility that the flow of cambial activity from the shoot may actually be guided along the root in the distal direction, as a result of the re-orientation of the tissues around the vascular peg at the base of the sucker bud from which the shoot arises. In other words, the phenomenon could conceivably be explained, in a very simple manner, without postulating polarity. On the other hand, it should be remembered that there are a number of phenomena indicative of polarity of cambial activity that could not be so explained.

Experiments and Results

Part 1. Extent of Development of Cambial Activity in the Proximal Direction in Roots Under Experimental Conditions

The material used was aspen poplar, *Populus tremuloides* Michx., and three different experiments, A, B and C (Fig. 1), were performed. In Experiment A the root was completely ringed some distance proximal to the sucker shoot,

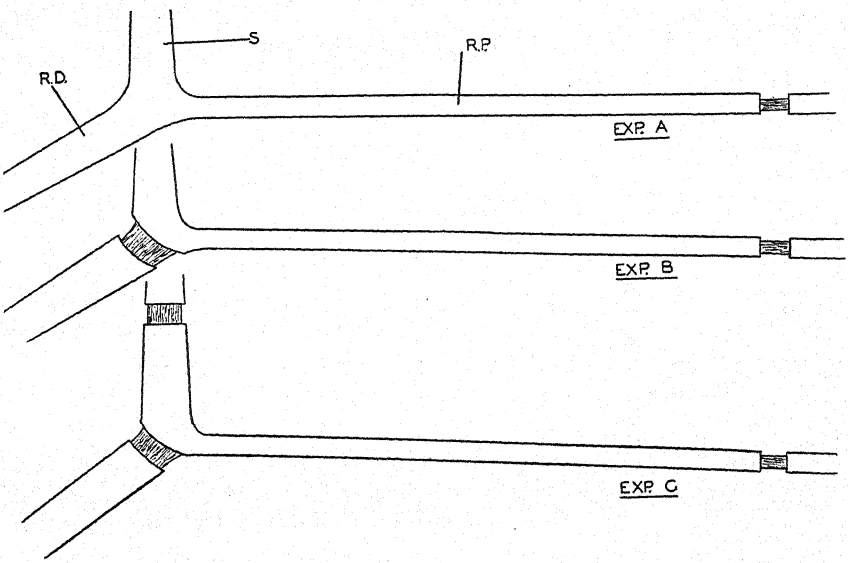

Fig. 1. *Illustrating the relative positions of the complete rings in Experiments* A, B *and* C. S = *the sucker shoot,* R.D. = *the root distal to the shoot, and* R.P. = *the root proximal to the shoot.*

in such a way as to remove all the tissues external to the xylem. In Experiment B, two complete rings were made, one proximal to the sucker shoot just as in Experiment A, and one immediately distal to the sucker shoot. In Experiment C three rings were made, one some distance proximal to the sucker, one immediately distal, and the third a short distance up the shoot. All growing points, if any, below the stem ring were removed. It was found that unless the ring was made quite close to the base of the stem, death of the parts above the ring ensued comparatively soon, whereas when the stem ring was near the base, the parts above could remain alive until the end of the growing season. In these experiments, the distance between the crotch and the proximal ring varied from 6 to 40 inches and was determined simply by the habit of the material. The sucker shoots varied in age from 5 to 14 years, and the operations were carried out within the last two weeks in May and the first week in June, in the years 1934 and 1935. At that time new xylem formation had not commenced in the roots. In all, about 100 trees were treated. The roots were uncovered as carefully as possible, and the operations performed on the trees as they occurred *in situ*. The exposed xylem in the rings was thoroughly scraped with a scalpel and the wound then rubbed with vaseline and finally covered with grafting wax. No healing over of the wounds ever took place. Usually the exposed roots were covered with loose turf after the operation. There was a marked tendency for sucker buds to arise just distal to the rings in the root, particularly the ring proximal to the sucker shoot. This tendency was not so evident just distal to the ring immediately distal to the shoot. These buds were usually removed whenever they were detected.

Subsequent growth of the treated trees was quite normal in the case of Experiment A. In B- and C-treated plants, the leaves tended to be smaller than usual and took on a bronze coloration at an early date. However, it was not at all uncommon to find trees receiving the B treatment perfectly normal in appearance with regard to leaf size and color. No difference in time was observed with respect to leaf fall at the end of the season. A few A- and B-treated specimens were left to overwinter and in the following spring they all leafed out at the same time as untreated trees. In most of the A-treated trees which had overwintered, the root proximal to the sucker was found to have died, either during the winter or early in the second season. Otherwise, the A-treated trees were perfectly normal during the second season. In B material the leaves were smaller, and bronzed early in all the specimens that had overwintered, and there appeared to be a definite reduction in extension growth during the second season. The specimens were still alive on August 9, 1935, when all of them were collected.

With regard to cambial activity, estimated in terms of xylem formation, the results can be summed up as follows: A falling gradient of xylem formation in the root on the side *proximal* to the sucker shoot was obtained in all three experiments. It was least marked in C material and most marked in B, while A was intermediate. The following estimations of the width of the

annual ring at different distances from the sucker shoot in four experiments, A 14(24 in.), A 16(16 in.), B 3(14 in.) and B 13(37 in.) (Tables I and II), will indicate the type of xylem gradient obtained in Experiments A and B. The figure in brackets after the number of the experiment indicates the length between the shoot-root crotch and the ring proximal to the crotch. All widths are compared with the width of the annual-growth ring for the current year in the shoot at a distance of six inches from its base, and this is arbitrarily rated at 100 in all cases. In no experiment were there any growing points on the shoot lower than six inches from its base. The actual widths of the annual ring in the shoot, six inches from the base, in A 16, A 14, B 3 and B 13, were, in arbitrary units, 140, 40, 32 and 37 respectively. It will be observed that there was very little difference between the widths in A 14, B 3 and B 13, so that the figures for these three experiments can be compared with one another in terms of actual width without any great error.

TABLE I

EXPERIMENTS A 16 AND A 14

		Width of annual ring	
		A 16	A 14
Stem:	6 in. from base	100	100
Root:	1 in. distal to shoot	150	136
Root:	6 in. distal to shoot	150	69
Root:	1 in. proximal to shoot	63	36
Root:	3 in. proximal to shoot	49	29
Root:	6 in. proximal to shoot	38	26
Root:	9 in. proximal to shoot	30	22
Root:	12 in. proximal to shoot	25	19
Root:	15 in. proximal to shoot	15	16
Root:	18 in. proximal to shoot		13
Root:	21 in. proximal to shoot		10
Treated		3/6/35	3/6/35
Collected		9/8/35	4/9/35

TABLE II

EXPERIMENTS B 3 AND B 13

		Width of annual ring	
		B 3	B 13
Stem:	6 in. from base.....	100	100
Root:	1 in. proximal to shoot	150	324
Root:	3 in. proximal to shoot	103	216
Root:	6 in. proximal to shoot	75	108
Root:	9 in. proximal to shoot	58	117
Root:	12 in. proximal to shoot	50	102
Root:	15 in. proximal to shoot		95
Root:	18 in. proximal to shoot		95
Root:	21 in. proximal to shoot		102
Root:	24 in. proximal to shoot		102
Root:	27 in. proximal to shoot		87
Root:	30 in. proximal to shoot		70
Root:	33 in. proximal to shoot		50
Root:	35 in. proximal to shoot		27
Treated		29/5/35	3/6/35
Collected		9/8/35	9/8/35

It is clear from the above results that a very appreciable gradient of cambial activity was laid down in the root *proximal* to the sucker shoot, in a system otherwise untreated except for a complete ring some distance proximal to the shoot (Experiments A 16 and A 14). There was no question of any stimulus passing across the ring, for precisely the same result obtained if the root was cut through completely, instead of ringed. Apparently, there-

ore, cambial activity is not rigidly polar in its development in the root. In Experiment B the gradient of xylem, laid down in the root on the proximal side, was not less and usually greater than that found on the distal side in untreated, or A-treated material. On the whole, it was found that the gradient on the proximal side in B material fell off rather more rapidly than it did on the distal side in A material. There was no indication whatsoever of any re-orientation of the tissues in the shoot-root crotch, despite the fact that the distal ring was shaped (Fig. 1), with the aim in view of facilitating any changes that might tend to take place.

Now it is well known that a basifugal development of cambial activity takes place locally from the upper edge of a complete ring in a shoot, quite independent of developing buds or elongation growth. Precisely the same sort of behavior occurs in roots, and Experiment C yielded information on this point. It will suffice to state that the gradient of xylem formation obtained in the proximal direction was exceedingly feeble. Several rows of xylem elements might be laid down just proximal to the shoot, but at a very short distance proximal, about 4 inches, the gradient practically faded out. The amount of xylem laid down proximal to the shoot in B material was, without a doubt, much greater than could be accounted for, if it were compounded simply of the amount of xylem formed as a result of A treatment plus the development resulting from C treatment.

A word now about the anatomical features of the new xylem laid down proximally in the root in these experiments. Almost invariably in Experiments A and B, the xylem in the root was denser than that of previous years. This was due principally to the fact that the vessels were smaller. Indeed, the new wood rather resembled typical stem wood. Occasionally a little parenchyma was found, formed at the beginning of cambial activity, but the new wood was not at all characterized by the presence of parenchyma as a constant feature, except towards the end of the gradient where it approached the ring proximal to the shoot. Here the wood invariably did include an abundance of parenchyma. Otherwise the wood laid down proximally was quite normal, consisting of vessels, tracheids, fibres and medullary rays. In Experiment C the new xylem did appear to be rather atypical. The conducting elements were again smaller in diameter, they often occurred in groups and consisted chiefly of tracheids rather than vessels, which although present were rather scarce. Moreover, the fibres were not thickened to nearly the same extent as in Experiments A and B. The wood laid down immediately distal to the shoot in normal untreated roots is usually rather denser and more like stem wood than that found at points further removed from the shoot, but it was observed in Experiment A, that the current year's wood distal to the shoot was not infrequently denser than that laid down in previous years. Moreover, one occasionally did find cases of A and B experiments, where the wood formed in the root proximal to the shoot was not markedly denser than the previously formed wood, particularly beyond the immediate vicinity of the shoot. It is quite possible that the formation of

denser wood in the roots in these experiments was determined to a certain extent by some such factor as water supply. Many of the feeder roots were inevitably destroyed during excavation prior to operation, and normal translocation of water would very probably be upset as a result of ringing, particularly in Experiments B and C. The wood laid down in the shoot in Experiments A and B was quite typical and did not appear to differ at all from that of previous years. A point of considerable interest is that the width of the post-operative growth ring in the shoot was sometimes greater and sometimes less than that of the previous year in both A and B experiments. In B material however, there was a marked decrease in width of the growth ring in the second season after operation, *i.e.*, in trees that had overwintered.

In Fig. 4; A, B and C illustrate the type of development obtained in three experiments, A1, B23 and C1. They show clearly that the wood formed in the root proximal to the shoot after the operative procedure is markedly denser than that of previous years. Moreover they give some idea of the extent of development that can be obtained in Experiments A and B at a considerable distance, seven inches, proximal to the shoot-root crotch. In C1 the section illustrated was cut at slightly less than one inch from the crotch; at a distance of seven inches from the crotch no new xylem had been formed. It was found, in all the B experiments, that for at least a considerable distance proximal to the shoot, the current year's growth ring was wider than any other growth ring laid down in that region previous to the operation. This applied only to the first post-operative growth ring. As has already been pointed out, development in the second season after operation was rather feeble. In Experiment A1 (Fig. 4, A), the current year's growth ring is wider than any of the others shown, but not as wide as some of the rings nearer the centre of the section and not included in the photograph. The only generalization that can be made with regard to the A experiments is that the width of the growth ring formed after the operation was not always less than that of previously formed rings. As far as the writer is aware, cambial activity in the proximal or morphologically upward direction has not hitherto been observed to the extent obtained in either Experiment A or B, with roots of poplar.

Part 2. Tissue Orientations in Relation to a Wound When Cambial Development Is Proximal in the Root

In a woody stem, if instead of making a complete ring a longitudinal phloem bridge is left, it is found that cambial activity accumulates or "piles up" immediately above or distal to the wound, flows through the bridge and then spreads out downwards and obliquely round the stem below the wound (Fig. 2). Transverse sections just above the wound show the xylem to be cut transversely at all points, except where the elements turn in slightly towards the bridge where they may be cut very slightly obliquely, whereas just below the wound the xylem is cut transversely only in the same longitudinal line as the bridge, and definitely obliquely at all other points. It is not proposed to discuss this type of behavior in any detail in this paper; what is of particular

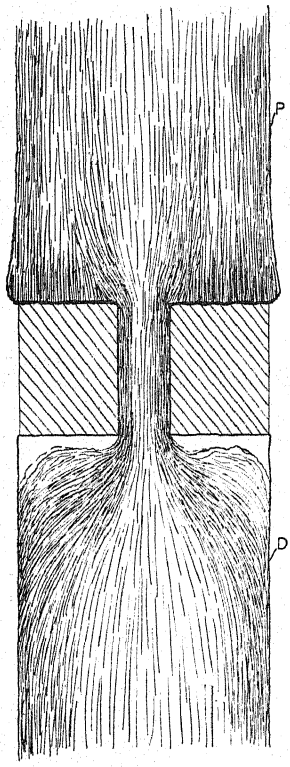

FIG. 2. *Tissue orientations in the wood of a shoot or root in relation to a ring with a longitudinal phloem bridge remaining.* P = *the root proximal to the wound or the shoot above the wound,* D = *the root distal to the wound or the shoot below the wound.*

inches from the crotch and the phloem bridge was somewhat less than one inch long. Note that the point seven inches proximal to the shoot was really distal to the wound, and the point eight inches proximal to the shoot really proximal to the wound, in terms of location on the root.

It is clear from the above analysis that wounding has interest is that it may be considered to be a typically polar phenomenon. Precisely the same type of behavior obtains in similarly wounded roots. The accumulation or "piling up" of xylem formation occurs on the proximal side of the wound, and the spreading out on the distal side. Wounds of this type were accordingly made in Experiments A and B, in that region of the root between the sucker shoot and the proximal complete ring, at a time when the cambium was still active and after a considerable gradient of xylem formation had been laid down in the proximal direction. *In every case the orientation of the tissues subsequently formed in relation to the wound was absolutely normal. There was no reversal either in Experiments A or B.* The following analysis of Experiment A 5 illustrates the type of result obtained. There were two dates of treatment, the first when the complete ring proximal to the shoot was made, and the second when the wound with the longitudinal phloem bridge was made. The second wound was located at about seven

TABLE III

EXPERIMENT A 5

(1st treatment, 12/5/34) (2nd treatment, 3/7/34)
(Collected, 23/7/34)

	Width of annual ring
Stem: 3 in. from base	100
Root: 2 in. distal to shoot	112
Root: 6 in. distal to shoot	93
Root: 1 in. proximal to shoot	39
Root: 2 in. proximal to shoot	35
Root: 3 in. proximal to shoot	32
Root: 4 in. proximal to shoot	31 + 0 to 7
Root: 5 in. proximal to shoot	27 + 7 to 14
Root: 6 in. proximal to shoot	24 + 14 to 17
Root: 7 in. proximal to shoot	23 + 1 to 27*
Root: 7.5 in. proximal to shoot	21 + 37†
Root: 8 in. proximal to shoot	21 + 20 to 27**
Root: 9 in. proximal to shoot	19 + 13
Root: 10 in. proximal to shoot	27 (=18 + 9)
Root: 11 in. proximal to shoot	19 (=16 + 3)
Root: 12 in. proximal to shoot	15

**Immediately distal to bridge.*
†Within bridge.
***Immediately proximal to bridge.*

brought about a marked increase in cambial activity in the vicinity of the wound. Moreover, the first xylem formed after wounding consisted largely of vessels, so that what was really a false growth-ring developed, enabling one to measure the extent of development prior to and subsequent to wounding. A few inches proximal to the wound, the demarcation between the wood formed prior to wounding and that formed after was not clear (this seems to be a constant feature), and the figures in brackets at 10 and 11 inches proximal to the shoot are rough extrapolations. As has already been stated, the tissue orientations around the wound were quite normal, no reversal having taken place. This is clearly indicated in the figures. Just proximal to the phloem bridge, the new wood laid down after wounding was but slightly excentric (20–27) and the elements were all cut transversely, whereas the new wood just distal to the bridge was very markedly excentric (1–27) and was cut transversely only in the same longitudinal line as the bridge, and obliquely at all other points as it spread downwards and obliquely round the root. The width of the new wood laid down in the bridge itself subsequent to wounding was always greater than that immediately distal or proximal to the wound. This was a constant feature. Precisely the same results were obtained in the B experiments. Fig. 4, D depicts a transverse section of the root within the bridge in Experiment B 2, and shows the extent of development prior to and subsequent to wounding at the time of collection. The results were definite and absolutely invariable, even when the wound was little more than one inch from the crotch. Moreover, the same result was obtained in four B experiments, with trees that had overwintered and were in their second season of growth after the first operative treatment. Here again, there was no reversal of the orientation of the tissues around the wound with the longitudinal phloem bridge. Whether similar results would be obtained in the third season of growth is not known. B material did rather poorly in its second season. There was less extension growth, fewer leaves produced, and a marked decrease in the amount of cambial activity. No experiments were performed with A material in the second season. Of the few that were left to overwinter, in most cases the root proximal to the sucker had died or was otherwise unsuitable for further treatment. The wood formed subsequent to wounding in these experiments was apparently quite normal. In Experiment B 2 (Fig. 4, D), the wood formed after wounding was somewhat denser than that laid down before wounding, but this was not always the case.

The complication arising from the above results is probably obvious. It is simply this, that there was no reversal of the tissue orientations around the wound with the longitudinal phloem bridge, under conditions where such a reversal might reasonably have been expected. The development of cambial activity is normally basipetal in the shoot and acropetal in the root, and the orientation of the tissues with relation to a wound with a longitudinal phloem bridge, in an otherwise untreated shoot or root (Fig. 2), could very naturally be interpreted as simply a manifestation of the normal mode of development.

From this point of view therefore, the fact that there was no reversal in Experiments A and B is rather surprising. There is however at least one point that may be of considerable significance in this connection. If a wound of the type under consideration is made in a dormant shoot or root, cambial activity is initiated locally around the wound, quite independent of cambial activity emanating from developing buds, which may indeed be entirely removed. Moreover, the new tissues orientate themselves in the vicinity of the wound precisely in the manner described above. Apparently to get cambial activity as a result of wounding, the phloem must be cut in such a way as to cause discontinuity of the elements. Any transverse or oblique incision would bring this about, whereas a longitudinal incision possibly would not if the elements were running strictly longitudinally. In addition, cambial activity only occurs if the cut has phloem tissue immediately distal to it in the stem, or proximal to it in the case of the root. This is clearly expressed in the fact that, if a complete ring is made in a stem, cambial activity leads to the production of a *basifugal* gradient of xylem from the upper margin of the ring, whereas no cambial activity is evident at the lower margin. It is, therefore, not inconceivable that the orientation of the tissues superimposed upon the proximal gradient in Experiments A and B was governed by the phloem tissue formed before the first operative procedure, despite the fact that a certain amount of new phloem had also been laid down, presumably in the same manner as the xylem, in a proximal gradient. But on the other hand, there was no evidence that the *amount* of cambial activity, superimposed upon the proximal gradient in the vicinity of the wound with the longitudinal phloem bridge, was determined by the phloem formed previous to the first operative procedure, since it was always greater relative to the amount of cambial activity obtained in the C experiments.

Part 3. Relationship Between Cambial Activity and Growth of Sucker Buds, in Regions of the Root Where the Normal Distal Development of Cambial Activity Is Not Taking Place

During the course of the above experiments, the writer was able to make observations of marked significance with relation to the behavior of sucker buds, which, if they did not arise definitely after, at least had not made vascular connection with the root xylem prior to, the operative procedure. These observations apply to such buds arising distal to the distal complete ring in Experiment B, or between two complete rings in regions of the root having no phloem connection with sucker shoots, as in Experiment C, and making vascular connection with the parent root in regions where the cambium was not dividing and the normal acropetal flow of cambial activity not present. Fig. 3 illustrates the manner in which the new xylem resulting from bud activity is laid down upon the surface of the wood of the previous year, as observed in pieces of root which had been peeled and allowed to dry out. Generally speaking, the new wood runs acropetally downwards and obliquely round the surface of the old wood. But just proximal to the bud, the new

wood actually runs almost directly proximal for a short distance before it turns. The writer has already pointed out that sucker buds tended to arise just distal to a complete ring, but wishes to emphasize that the behavior illustrated was commonly observed at points considerably removed from any complete ring. Behavior of similar buds close to a complete ring was, however, the same. Precisely the same type of behavior was observed close to the complete ring proximal to the shoot in Experiments A and B, again with buds that had not attained vascular connection with the root prior to operation. Here, surprisingly enough, the wood resulting from bud activity spread distally or acropetally upon the surface of wood that had been, or was being laid down in a gradient in the opposite or proximal direction. It is true, however, that these buds were always observed very close to the complete ring, *i.e.*, right at the end of the proximal gradient where the xylem contained an abundance of parenchyma.

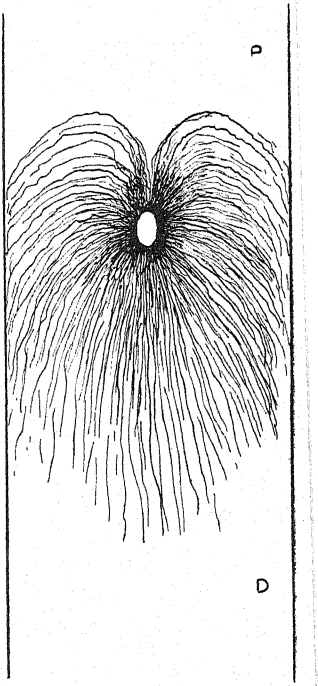

FIG. 3. *Illustrating the way in which cambial activity emanating from developing buds spreads over the surface of the previously formed wood, in regions of the root where the normal acropetal flow of cambial activity is not present.* P = *the root proximal to the bud,* D = *the root distal to the bud.*

A point of interest arising at this time is related to a former statement by the writer (1), to the effect, that the definite "flow pattern" in the root xylem below the sucker bud under normal conditions is a manifestation of the reaction between root cambial activity and the obstruction offered by the vascular peg at the base of the sucker bud. In view of the above observations, bud activity of itself could not bring about the formation of such a pattern, and so the writer's earlier statement receives some support.

PART 4. SIMILAR EXPERIMENTS WITH POPLAR STEMS

The reader will probably be able, without much difficulty, to picture experiments with stems, essentially similar to those already described with roots. Instead of the shoot-root crotch we have now to consider the branch crotch. Corresponding to Experiment A, one complete ring was made in one member of the crotch and this limb was completely disbudded below the ring. The other limb was untreated. Corresponding to Experiment B, a second complete ring was made immediately below the crotch, and corresponding to Experiment C, three complete rings were made, one on each of the two limbs and another immediately below the crotch. The material between the rings was completely disbudded. Ringing was carried out during the month of April, before the advent of bud break. The results will be

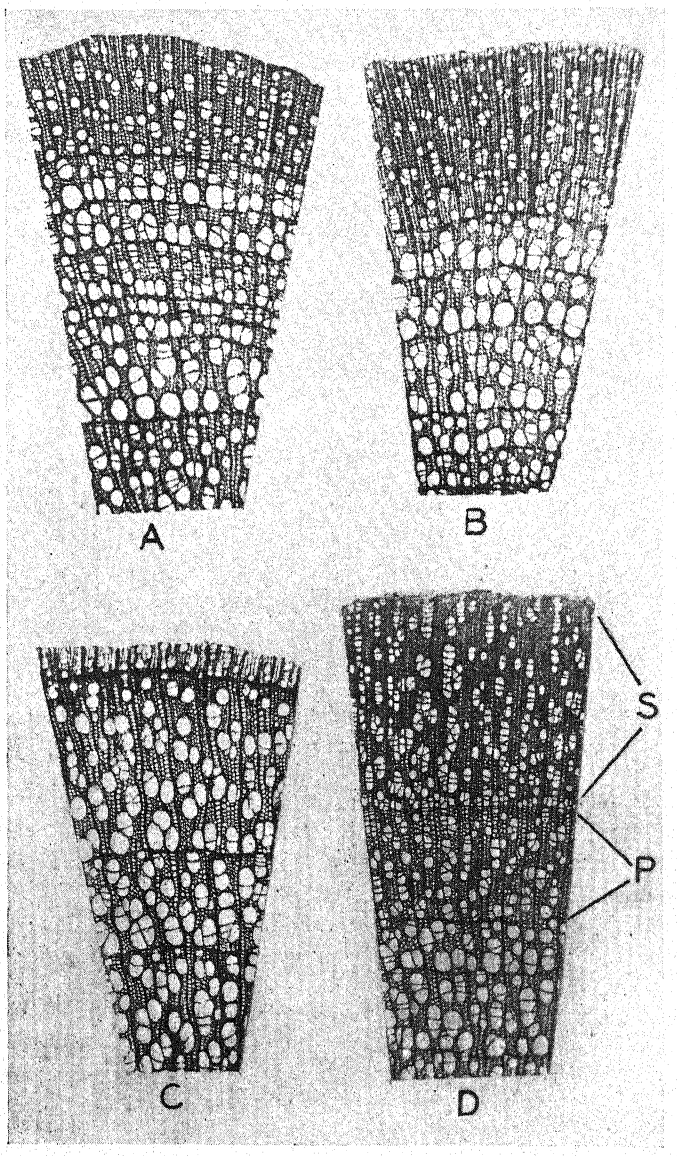

FIG. 4. A. *Transverse section of root of aspen poplar, seven inches proximal to the shoot in Experiment A 1 (13″). Treated 16/5/34, collected 13/8/34. The outermost ring is the post-operative growth ring.* × 24. B. *Transverse section of root of aspen poplar, seven inches proximal to the shoot in Experiment B 23 (13″). Treated 15/5/34, collected 13/8/34. The outermost ring is the post-operative growth ring.* × 24. C. *Transverse section of root of aspen poplar, slightly less than one inch proximal to the shoot in Experiment C 1 (14″). Treated 16/5/34, collected 13/8/34. The outermost ring, which is very narrow, is the post-operative growth ring.* × 24. D. *Transverse section of root of aspen poplar in Experiment B 2 (30″). Treated 15/5/34, collected 13/8/34. On 3/7/34 a ring with a longitudinal phloem bridge remaining was made in the root about nine inches proximal to the shoot, and the section illustrated was cut within the bridge. The xylem formed prior to and subsequent to wounding (3/7/34) is indicated by P and S respectively.* × 24.

NOTE.—*The figure in brackets after the number of the experiment in the above, indicates the length in inches between the shoot-root crotch and the proximal complete ring.*

treated as briefly as possible and they all lead to the same conclusion; *viz.* that cambial activity in any but the basipetal direction was extremely limited in extent. In Stem Experiment A, the new wood formed in the untreated limb flowed round the base of the ringed limb in a loop, and sometimes there was no basifugal or acropetal development of cambial activity in the ringed limb. Usually, however, there was a slight development acropetally, and occasionally one did find an acropetal development for a few inches above the crotch, in the ringed limb. But at the most, the extent of development was very small. Stem Experiment A resembled rather closely the state of affairs obtaining in that method of pruning where a snag is left above the bud. Wray (12) has shown that, in such a case, cambial activity emanating from the bud flows downwards and obliquely around the stem, and there is no development in the acropetal direction in the snag. The snag, of course, dries out rapidly. Knight (5), on the other hand, observed a slight upward development of cambial activity in disbudded apple shoots, from the first growing branch below, and Sledge (7) reports that Swarbrick observed a very slow upward spread of new wood formation in a ringed apple shoot, from the first bud below, up to the lower edge of the ring.

In Stem Experiment B, cambial activity from the limb bearing developing growing points again tended to flow round the base of the ringed limb in a loop, but further progress was of course prevented by the complete ring below the crotch. The ultimate result was usually a marked "piling up" of new wood just above the crotch ring. This was particularly obvious in a thick band of wood running transversely round the base of the ringed limb. Cambial activity in the acropetal direction in the ringed limb was much the same as in Stem Experiment A. Sometimes there was none at all, usually there was a little, and occasionally cambial activity spread upwards for a few inches. But here again, cambial activity in the acropetal direction was at the most exceedingly feeble. In Stem Experiment C, a feeble gradient of cambial activity, spreading acropetally upwards in both limbs from the upper edge of the complete ring below the crotch, was the invariable result. Just above the crotch ring a very appreciable amount of xylem usually formed, but it was "piled up", and the gradient fell off very rapidly. Actually, Stem Experiment C yielded results very similar to Root Experiment C. There was, however, no comparison at all between the extent of development of cambial activity in Experiments A and B in stems, relative to that obtained in roots. In the stem experiments, all the tissues above a complete ring invariably died before the end of the growing season.

Discussion

In view of the mode of development of cambial activity emanating from sucker buds (arising after, or at all events having no vascular connection with the parent root prior to the operative procedure, and in regions of the root where the normal acropetal flow of cambial activity is not present) it is to be concluded that the marked development of cambial activity distal to a sucker

shoot, relative to that on the proximal side, is not determined by a previous re-orientation of the tissues at the base of the sucker bud. The answer, it would appear, is to be looked for in polarity of cambial development, and in this connection a number of points arise for discussion.

Jost (3, 4) was the first investigator to observe that cambial activity in the stem travels only in the basipetal direction. It has also been pointed out at various times by Priestley (6), by Wray (12) by Snow (8, 9, 10) and a few others. Snow (10) also reports that cambial activity develops only in the morphologically downward direction in roots of *Vicia Faba*. In fact this polar mode of development of cambial activity is now accepted as a general rule, with but few exceptions. However it is with the exceptions that the writer is mainly concerned. The observations of Knight and Swarbrick, of a feeble basifugal development of cambial activity in apple stems under certain conditions, have already been mentioned. These observations are confirmed by the writer's results with poplar stems. Here of course, the upward development of cambial activity is admittedly very feeble. However, in Root Experiment A, the development of cambial activity in the proximal direction is by no means feeble, and on the basis of this experiment alone, it would seem that polarity of cambial activity in poplar roots is very definitely not rigid or unconditional, and that any definition of polarity must be couched in terms of a *tendency* to develop in the distal rather than in the proximal direction. Fundamentally this may also apply to cambial activity in stems. The marked difference in the extent of development basifugally in stems, relative to that proximally in similar experiments with roots, might be conditioned by other factors, for example, water supply. A reasonable explanation of the results obtained in Root Experiment B is that the cambial stimulus that would normally travel acropetally is diverted proximally, which would also support a definition of polarity in terms of a tendency. The mode of development of new wood emanating from developing buds under the conditions previously described (Fig. 3) is of great interest. Actually, cambial activity at first seems to be inherently capable of developing in all directions possible, distally, laterally and proximally, and it looks almost as if the ultimate development distally were being conditioned by some factor not inherent in cambial activity as such.

Reference might also be made to the local basifugal development of cambial activity from the upper edge of a complete ring in stems, and the corresponding development proximal to a complete ring in roots. These are widely recognized exceptions to the general rule in regard to cambial development. But of still greater interest is the local development of cambial activity in the vicinity of a wound, where instead of a complete ring being made, a longitudinal phloem bridge is left. In the stem, there occurs a basifugal development of xylem formation from the upper edge of the wound, similar to that obtained when the ring is complete. Within the bridge, however, development is basipetal, and just below the bridge it is basipetal and lateral. A correspond-

ing type of development obtains in similarly treated roots. These remarks apply to cambial activity in the vicinity of wounds, independent of any normal cambial activity emanating from developing shoots. A more detailed account of cambial activity in relation to wounding will be published later.

Some recent observations by Elliott (2) are also worthy of mention. He has shown that in *Acer* cambial activity proceeds acropetally into the leaf from the junction of the lamina and petiole, and in *Castanea* acropetally along the petiole from its base into the lamina. At the same time there is the usual basipetal development of cambial activity down the shoot from the top of the petiole in *Acer* and from the bottom of the petiole in *Castanea*.

Now there is a possible danger of the idea of *rigid* polarity of cambial activity becoming almost a general law in the minds of some investigators, particularly so in view of the fact that Snow and LeFanu (11) have shown that an ether extract of urine promotes cambial activity, and the possibility that the hormone promoting cambial activity may be identical with the auxin causing cell elongation in the oat coleoptile, in which according to a number of investigators translocation of the auxin is strictly polar. The writer suggests in this connection that the fact that cambial activity does not or apparently cannot proceed in any but the morphologically downward direction, in some cases, does not necessarily mean that it cannot possibly develop in the opposite direction under any condition.

The fact that there was no reversal of the tissue orientations in relation to the wound with the longitudinal phloem bridge, made subsequent to the first operative procedure in Root Experiments A and B, is very puzzling. However, a possible explanation has been indicated and at all events the writer does not feel that his conclusion, that cambial activity is not rigidly and unconditionally polar, is rendered untenable because of the phenomenon just mentioned.

Recognition of the fact that polarity of cambial activity, which is but one of many polar phenomena in plants, constitutes a difficult problem is of course not new, and no pretence is made that the foregoing experiments indicate a solution of polarity of cambial activity in particular. However, definite experimental results have been obtained, on the basis of which the following conclusion is submitted for consideration; *viz.*, that cambial activity is polar in its development, when polarity is defined in terms of a *tendency* to develop in the morphologically downward direction, rather than in the morphologically upward direction. On the other hand, it may be suggested that cambial activity as a process is not inherently polar in its development, but is determined by some other factor. If however, this determining factor should be polarity of the organism as a whole, or polarity of some particular organ as a whole, then the two interpretations really become identical, and polarity of the organ or organism as a whole would have to be defined in terms similar to those suggested for cambial activity.

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STRIPE RUST, *PUCCINIA GLUMARUM*, IN CANADA[1]

By Margaret Newton[2] and T. Johnson[3]

Abstract

A study was made of the distribution of *Puccinia glumarum* (Schm.) Erikss. and Henn. in Canada, its specialization, host range, and reaction to environmental conditions. Unlike *Puccinia graminis* Pers., this rust has a limited distribution, being confined to British Columbia, Alberta, and the western half of Saskatchewan. The natural hosts include a number of native grasses, particularly *Hordeum jubatum* L. and certain species of *Agropyron*, *Elymus*, and *Bromus*. Wheat and barley also become infected although to a rather limited extent. Stripe rust collected on the above-mentioned hosts has been studied in the greenhouse and has in all cases shown ability to attack wheat varieties. In all instances where identification of physiologic forms was carried out the rust strains were classified as either form 8 or form 13 of wheat stripe rust, the latter form being the more common. The fact that the present authors have collected known physiologic forms of wheat stripe rust on species of *Hordeum*, *Elymus* and *Agropyron*, and have shown that forms 4, 6, 8 and 13 can attack seedling plants of *Hordeum*, *Agropyron*, and *Elymus* species throw a doubt on the existence of the *Hordei*, *Elymi*, and *Agropyri* varieties created by Eriksson.

Greenhouse studies showed that *P glumarum* is extremely sensitive to environmental conditions, particularly temperature. The optimum for uredospore germination is 10° to 12° C., and for rust development 13° to 16° C. Varieties susceptible at from 10° to 16° C. developed resistance at higher temperatures, becoming extremely resistant at 25° C. On account of the sensitiveness of this rust to high temperatures it seems improbable that it will ever become thoroughly established in Manitoba and Saskatchewan, as in these two provinces the summer temperature is probably too high to permit its development.

Introduction

Stripe rust, *Puccinia glumarum* (Schm.) Erikss. and Henn., has a more limited distribution in Canada than the other cereal rusts, being confined to British Columbia, Alberta, and the western half of Saskatchewan. Collections have been made as far east as Whitewood, Saskatchewan, which is 102° W. longitude, or a little beyond the eastern limit reported for this rust in the United States by Humphrey *et al* (13). Some concern has been felt in Canada lest this rust might be slowly advancing eastward and that it would be merely a matter of time until it would become established throughout the prairie provinces of Canada. It seemed advisable, therefore, to ascertain what environmental factors influenced the spread of the organism, and whether or not more than one physiologic form of the organism was present. This study was begun in July, 1927.

Discovery and Host Range

In Canada *Puccinia glumarum* was first discovered by Fraser (5) in 1918, at Edmonton, Alberta, on *Hordeum jubatum* L. In 1924 it was reported on barley by Fraser and Conners (5) and in 1926 it was found on a number of wheat varieties by Sanford (24).

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Collections of the rust have been made, chiefly in British Columbia, by the present authors on the following species of grasses: *Agropyron repens* (L.) Beauv., *A. trachycaulum* (Link) Malte var. *unilaterale* (Vasey) Malte, [*A. Richardsonii* (Trin.) Schrad.], *A. Smithii* Rydb., *A. trachycaulum* (Link) Malte var. *typicum* Fernald [*A. tenerum* Vasey], *Bromus carinatus* Hook. & Arn. var. *Hookerianus* (Thurb.) Sheer, *B. ciliatus* L., *B. marginatus* Nees, *B. sitchensis* Bong., *Elymus glaucus* Buckley, *E. virescens* Piper, *Hordeum jubatum* L. var. *caespitosum* (Scribn.) Hitchc., and *H. jubatum* L. Several of the above-mentioned species have also been reported as hosts of stripe rust in Alberta by Sanford and Broadfoot (25, 27) who also include as hosts *A. trachycaulum* (Link) Malte [*A. caninum* (L.) Beauv.], *A. cristatum* (L.) *Gaertn.*, *A. Dagnae* Grossh.*, *A. dasystachyum* (Hook.) Scribn., *A. desertorum* Schult.*, *A. rigidum* Beauv. [*A. elongatum* Host.] *A. Griffithsii* Scribn. & Smith, *A. pungens* Roem. & Schult*, *A. sibericum* (W.) Eichw.*, *A. spicatum* (Pursh.) Scribn. & Smith.

Studies on the Effect of Environmental Factors Upon the Spread of *Puccinia glumarum* in Western Canada

At the beginning of this study difficulties were encountered in establishing cultures of *Puccinia glumarum* in the greenhouse. Plants inoculated during the hot summer months flecked heavily but developed no pustules. Those inoculated in the autumn, when the temperature in the greenhouse was considerably lower than in the summer months, became heavily infected. It seemed fairly evident that the high temperature prevailing during the summer months was in some way responsible for the failure of the plants to become infected. An attempt was made, therefore, to determine the optimum temperature, for the germination of uredospores and for the best development of the organism within the tissues of the host plant.

Influence of Temperature upon the Germination of Uredospores

Uredospores freshly formed in the greenhouse on seedling leaves were germinated in hanging drops of tap water in Van Tieghem cells and in Syracuse watch glasses at six different temperatures ranging from a lower limit of from 2° to 3° C. to an upper limit of from 22° to 25° C. (Table I). At each temperature, from 2000 to 4000 spores were counted. Comparative tests were also made with uredospores of *Puccinia graminis Tritici* Erikss. and Henn. at the same temperatures.

In all the tests the best germination of the stripe-rust spores was consistently obtained at 10° to 12° C., while below 5° C. and above 20° C. there was a sharp decline in germination. It will be noticed that the germination percentages given in Table I are considerably lower than those of stem rust at all except the lowest temperature (2° to 3° C.). The germination percentages are, however, somewhat higher than those obtained by Raeder and Bever for *P. glumarum* (22), but lower than those reported by Wilhelm (34). The

* *Species not native and probably not widespread in Western Canada.*

TABLE I

A COMPARISON OF THE GERMINATION OF NEWLY FORMED UREDOSPORES OF *Puccinia glumarum* AND *Puccinia graminis Tritici*

Temperature, °C.	*P. glumarum*		*P. graminis Tritici*	
	Number of tests	Germination, %	Number of tests	Germination %
2 – 3	24	12	13	tr.
5 – 7	10	46	5	83
10 – 12	15	59	8	77
14 – 16	14	39	10	77
20 – 22	27	tr.	6	89
22 – 25	28	tr.	6	69

former, however, made no attempt to germinate spores of the same age, whil the latter used spores that were all of approximately the same age. As th spores of *P. glumarum* are apparently very sensitive to environmental con ditions, it seems very probable that no two workers could possibly obtain th same results unless the spores were produced and germinated under identica conditions.

Not only were the uredospores of *P. glumarum* less viable than were thos of *P. graminis* but their range of temperature for germination appeared t be somewhat narrower. Good germination was secured only between 5° an 18° C. whereas the spores of *P. graminis Tritici* germinated freely betwee 5° and 25° C.

INFLUENCE OF TEMPERATURE AND HUMIDITY UPON THE LONGEVITY OF UREDOSPORES

During the hot summer months field cultures, approximately ten days old, from British Columbia and Alberta germinated very poorly, showing that the uredospores underwent a great loss of viability during transit. The sensitivity of uredospores of *P. glumarum* to environmental conditions has been commented upon by several investigators, Mehta (18) reported a germination of only 5% after storage for one month in the laboratory, and Wilhelm (34) stated that spores only eight days old showed a delay in germination and a loss of viability. Gassner and Straib (7) found that exposure of the spores of *P. glumarum* to direct sunlight at from 30° to 35° C. for four hours practically destroyed their viability but did not affect spores of *P. triticina* Erikss., *P. dispersa* Erikss., or *P. coronifera* Kleb.

The sensitiveness of the spores to external conditions and the difficulty of culturing the rust in the greenhouse during the three hot summer months made it imperative to devise a satisfactory method for preserving the cultures during the period unfavorable for cultural work. The experiments of Becker (2) had indicated 0° C. and 40% relative humidity as the optimum conditions of storage. Under these conditions she still found a portion of the spores germinable, at the end of 433 days. Raeder and Bever (22), however, found

the most satisfactory conditions for the storage of spores to be a temperature of from 9° to 13° C. and a relative humidity of 49%, under which conditions spores remained viable for a period of 88 days.

In the present study a number of tests were made to determine the longevity of uredospores of three physiologic forms of *P. glumarum* at a temperature of 5° C. and a relative humidity of 50%. The results of these tests are given in Table II which shows that at the end of 75 days from 3 to 7% of the spores germinated while a trace of germination was secured after 128 days. That this relatively weak germination sufficed to cause infection was shown by the successful infection of ten wheat seedlings with spores of each of four physiologic forms that had been in storage for 95 days. In a repetition of this experiment a year later, all cultures in storage germinated sufficiently well to cause infection at the end of three months. It would seem, therefore, that freshly formed uredial material of *P. glumarum* can be stored with safety for at least three months, a period corresponding to the critical midsummer period during which this rust can only be kept in culture in the greenhouse with extreme difficulty.

TABLE II

PERCENTAGE GERMINATION OF UREDOSPORES OF THREE PHYSIOLOGIC FORMS OF *Puccinia glumarum* STORED AT 5° C. AND 50% RELATIVE HUMIDITY FOR DIFFERENT PERIODS

(Germination based on a count of 1000 spores)

Physiologic form	No. of days uredospores in storage	Germination, %
13	60	14.6
	65	10.0
	70	4.0
	80	5.6
	90	3.6
	100	0
	110	trace
	150	0
	400	0
8	75	7.6
	80	2.0
	90	trace
	95	trace
	130	0
	400	0
4	65	4.9
	70	6.2
	75	3.4
	80	trace
	90	trace
	128	trace
	150	0
	400	0

INFLUENCE OF TEMPERATURE UPON UREDIAL DEVELOPMENT

It has long been known that the reactions of many rusts are influenced by changes in environment, particularly by changes in temperature and light intensity. Waterhouse (33), in studying a number of the cereal rusts, found that "major differences in the rust reactions may be brought about by altering the environmental conditions under which the tests are made. Complete susceptibility under summer conditions may change to complete resistance under winter conditions". Similar fluctuations due to temperature changes were noted by Johnson (15) in the types of infection upon wheat infected with

P. graminis Tritici, and by Gordon (11) and Peturson (21) upon oats when infected with *P. graminis Avenae* Erikss. and Henn. and *P. coronata* Cda. respectively.

Experiments were therefore conducted to ascertain to what extent temperature influences the rust reactions of wheat varieties to *P. glumarum*.

While the work was still in progress Gassner and Straib (6) published results which showed that wheat varieties susceptible to *P. glumarum* at low temperatures become resistant or immune at moderately high temperatures, results which agreed with our own observations (19).

Experimental Methods

The plants used for these experiments were grown in duplicate sets and kept at ordinary greenhouse temperature prior to inoculation. Inoculations were made when the plants were in the first-leaf stage. The methods of inoculating and culturing the rust, as well as the symbols used in recording the rust reactions, were, with certain modifications, similar to those described by Hungerford and Owens (14), which were, in turn, adaptations of those first used by Stakman and Piemeisel (29) for stem rust. The Arabic numerals "0" to "4" indicate the type of infection in order of increasing severity. A description of these infection types is as follows:

Resistant Class

Type 0(n) No uredia; dead necrotic areas often present.

Type 1 Uredia few or minute, generally surrounded by dead areas; portions of leaves sometimes killed or discolored.

Type 2 Uredia normal in appearance, but few and scattered; discoloration of leaf tissues common.

Susceptible Class

Type 3 Uredia normal, moderately abundant; little discoloration of leaf tissue.

Type 4 Uredia normal and very abundant, appearing uniformly over surface of inoculated leaf; no discoloration in early stages of infection.

The signs (=), (−), and (±), are used to indicate quantitative variations in the above types.

Influence of Two Different Temperatures Upon Infection Types

Nine wheat varieties, Acme, Arnautka, Little Club, Mindum, Spelmar, and Vernal were inoculated with *P. glumarum* forms 4, 8, and 13. One set of plants was kept at 25.3° C. and the other at 12.9° C. Above or below either of these temperatures, the variation was not more than 2°.

The results presented in Table III show that at 25.3° C. all the hosts tested to the three physiologic forms were completely resistant, while at 12.9° C.

TABLE III

THE RELATIVE REACTIONS OF WHEAT VARIETIES INOCULATED WITH *Puccinia glumarum*, FORMS 4, 8, AND 13, AND KEPT AT TWO DIFFERENT TEMPERATURES

Variety	Form	Number of plants inoculated	Number of plants infected	Reactions at	
				25.3° C.	12.9° C.
Little Club	4	20	20	Resistant	Susceptible
Spelmar	4	22	21	Resistant	Susceptible
Vernal	4	26	26	Resistant	Susceptible
Acme	8	21	18	Resistant	Susceptible
Arnautka	8	25	24	Resistant	Susceptible
Brevit	8	24	22	Resistant	Susceptible
Chul	8	22	22	Resistant	Susceptible
Mindum	8	26	23	Resistant	Susceptible
Prelude	8	26	25	Resistant	Susceptible
Vernal	8	20	20	Resistant	Susceptible
Acme	13	22	22	Resistant	Susceptible
Chul	13	24	23	Resistant	Susceptible
Mindum	13	23	22	Resistant	Susceptible
Prelude	13	24	24	Resistant	Susceptible

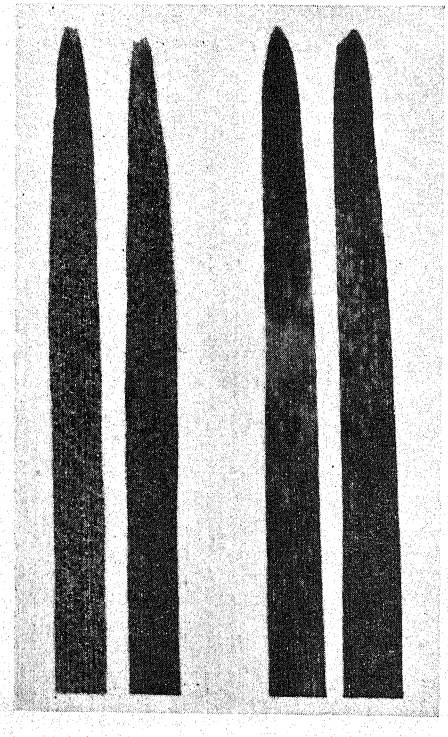

FIG. 1. *Reaction of Chul wheat to P. glumarum, form 13, at 12.9° C. (left) and at 25.3° C. (right).*

they were completely susceptible. At the high temperature (25.3° C.) the plants flecked heavily but produced practically no uredia, but at the low temperature (12.9° C.) abundant uredia were produced. The reaction of Chul wheat to form 13 at 25.3° C. and 12.9° C. is shown by means of a photograph (Fig. 1).

In order to ascertain if the mycelium in the heavily-flecked plants was still alive and capable of further development under cooler conditions, the heavily flecked plants were placed for ten days in a section of the greenhouse maintained at 12.9° C. Plants with only flecks developed no uredia, a result which showed that the mycelium had died at the high temperature (25.3° C.). Those with flecks plus minute uredia now developed normal-sized uredia. In these plants the mycelium was

apparently injured at a temperature of 25.3° C. but not completely destroyed. However, as only a small number of plants kept in the hot greenhouse (25.3° C.) ever bore uredia, it seems safe to assume that the mycelium of stripe rust is usually killed in plants kept continuously ten days or more at that temperature.

Effect of Temperature on Infection Type

(*a*) *When plants are kept for a definite number of days at one temperature and then transferred to another*

Some experiments were carried out to determine the effect of exposing wheat plants, which are susceptible to stripe rust, to a relatively high temperature before transferring them to a relatively low temperature or *vice versa*. In the month of March, 24 pots of Prelude wheat were inoculated with form 4. After removal from the incubation chambers, 13 of the pots were placed in a section of the greenhouse maintained at a temperature of 22.7° C. and 13 in a section at a temperature of 13.7° C. At the end of one day, and on each succeeding day for 11 days, one pot was transferred from the higher to the lower temperature, and one from the lower to the higher temperature. One pot was kept as a control in each section. The results indicate that the longer the infected wheat plants were kept at a temperature of 22.7° C. before they were transferred to a temperature of 13.7° C., the greater was their resistance (Table IV) and, conversely, the longer they were kept at a temperature of 13.7° C. before they were transferred to a temperature of 22.7° C., the greater was their susceptibility.

TABLE IV

THE TYPES OF INFECTION PRODUCED BY *Puccinia glumarum*, FORM 4, ON PRELUDE WHEAT WHEN PLANTS WERE KEPT FOR DIFFERENT NUMBERS OF DAYS AT A TEMPERATURE OF 22.7° C. AND THEN TRANSFERRED TO A TEMPERATURE OF 13.7° C.

Days at 22.7° C.	Days at 13.7° C.	Infection results	Reaction
1	11	$\frac{12}{12}$(4)	Susceptible
2	10	$\frac{7}{9}$(4) $\frac{1}{9}$(n)	Susceptible
3	9	$\frac{9}{10}$(3)	Susceptible
4	8	$\frac{5}{12}$(3) $\frac{4}{12}$(2) $\frac{2}{12}$(n)	Semi-resistant
5	7	$\frac{2}{8}$(3) $\frac{4}{8}$(2) $\frac{1}{8}$(n)	Semi-resistant
6	6	$\frac{3}{15}$(2) $\frac{3}{15}$(1)	Resistant
7	5	$\frac{1}{12}$(1) $\frac{9}{12}$(n)	Resistant
8	4	$\frac{12}{14}$(n)	Resistant
9	3	$\frac{16}{16}$(n)	Resistant
10	2	$\frac{9}{9}$(n)	Resistant
11	1	$\frac{14}{14}$(n)	Resistant
Kept continuously at 22.7° C.		$\frac{10}{11}$(n)	Resistant
Kept continuously at 13.7° C.		$\frac{9}{10}$(4)	Susceptible

Explanatory note.—The infection types are enclosed in brackets. The figures preceding the infection types indicate the number of leaves infected, e.g., $\frac{5}{12}$(3) $\frac{4}{12}$(2) $\frac{2}{12}$ (n) shows that 5 out of 12 leaves bore a (3) type of infection, 4 out of 12 leaves, a (2) type of infection, and 2 out of 12, necrotic flecks.

(b) When plants are kept for a definite number of hours each day at one temperature and then transferred to another

Since, however, the real object of these temperature studies was to find out whether stripe rust was likely to become prevalent in the great wheat-growing areas of Manitoba, Saskatchewan, and Alberta, some experiments were carried out to ascertain how many hours of high temperature the wheat plants could tolerate daily and still permit a normal type of infection to develop; for it seemed possible that, although during the growing season the night temperatures in Manitoba, Saskatchewan, and Alberta are always relatively low, the day temperatures are generally fairly high and might inhibit all stripe rust development.

Fourteen pots of Prelude wheat were inoculated with form 4 and placed in a greenhouse maintained at 12.9° C. They were carefully numbered and arranged in seven sets of two pots each. Set 1 was kept daily for 8 hours in this greenhouse and was then transferred for 16 hours to a greenhouse maintained at 25.3° C. This procedure was repeated for 17 days. Sets 2, 3, 4, and 5, were likewise transferred daily from one temperature to the other but for different lengths of time. The period during which any one of these sets was kept in either greenhouse is given in Table V. Sets 6 and 7 were used as controls. The former was kept continuously at 12.9° C. and the latter at 25.3° C. The experiment was repeated later using a different physiologic form, a different variety, and two other temperatures, with quite similar results.

TABLE V

THE TYPES OF INFECTION PRODUCED BY *Puccinia glumarum*, FORM 4, ON PRELUDE WHEAT WHEN PLANTS WERE KEPT FOR A DEFINITE NUMBER OF HOURS EACH DAY AT 12.9° C. AND THEN TRANSFERRED TO A TEMPERATURE OF 25.3° C. FOR THE REMAINDER OF THE DAY

Plants	Hours at		Infection Results	Reaction
	12.9° C.	25.3° C.		
Set 1	8	16	$\frac{22}{30}$(n)	Resistant
Set 2	10	14	$\frac{20}{24}$(n)	Resistant
Set 3	12	12	$\frac{1}{23}$(3) $\frac{18}{23}$(2) —	Resistant
Set 4	14	10	$\frac{12}{20}$(2) $\frac{8}{20}$(3) —	Semi-resistant
Set 5	16	8	$\frac{16}{21}$(4) —	Susceptible
Set 6	Kept continuously at 12.9° C.		$\frac{23}{23}$(4)	Susceptible
Set 7	Kept continuously at 25.3° C.		$\frac{17}{20}$(n)	Resistant

From Table V it will be seen that plants kept at (25.3° C.) for 12 hours or more daily became resistant while those for 8 hours or less were susceptible; the ones held at the higher temperature for 10 hours were intermediate in reaction, or semi-resistant.

A study was made of the temperature prevailing in the prairie provinces and British Columbia to ascertain if some parallel could be found between the behavior of the rust in the greenhouse at known temperatures and its development under field conditions. In Table VI are given the mean daily

TABLE VI

MEAN DAILY MAXIMUM AND MINIMUM TEMPERATURE, TOGETHER WITH AN AVERAGE OF THE EXTREME HIGHEST AND EXTREME LOWEST RECORDED FOR EACH MONTH, IN CERTAIN DISTRICTS IN BRITISH COLUMBIA, ALBERTA, SASKATCHEWAN, AND MANITOBA, DURING JULY AND AUGUST, FOR THE FIFTEEN YEAR PERIOD, 1919 TO 1933

District	Temperature, °F.							
	July				August			
	Mean daily max.	Mean daily min.	Mean extreme highest	Mean extreme lowest	Mean daily max.	Mean daily min.	Mean extreme highest	Mean extreme lowest
British Columbia								
Southeast Vancouver Island	72.3	51.7	93.0	39.7	72.7	51.7	93.9	41.0
Lower Fraser River	75.3	53.3	93.4	41.7	74.9	53.5	93.1	42.0
Alberta								
North Saskatchewan River	75.5	47.3	98.7	26.8	72.6	45.0	94.9	22.2
The Bow River	78.7	49.8	101.0	29.2	76.9	47.1	98.3	25.9
Saskatchewan								
Qu'Appelle River	79.3	51.7	103.5	32.9	77.4	48.4	100.1	28.4
South Saskatchewan River	81.0	51.3	100.1	32.0	78.7	48.5	98.3	28.7
North Saskatchewan River	77.8	49.6	98.5	31.9	75.4	46.6	94.9	27.5
The Saskatchewan Forks	78.8	52.3	98.1	35.1	76.3	48.5	96.1	30.4
Manitoba								
Qu'Appelle and Assiniboine Rivers	79.3	53.6	100.4	35.1	77.7	50.3	98.0	29.9
The Red River	79.8	55.4	97.5	34.9	78.2	52.1	97.7	30.1

The temperatures in this table are expressed in Fahrenheit, the scale used in Canadian meteorological records.

minimum and maximum temperatures for July and August, together with the average of the extreme highest and extreme lowest temperatures, in certain districts in British Columbia, Alberta, Saskatchewan, and Manitoba for the 15-year period 1919 to 1933.

It will be seen from Table VI that, in July as well as in August, the mean minimum daily temperature is somewhat lower in Alberta than in Saskatchewan, and lower in Saskatchewan than in Manitoba. For the same districts of the prairie provinces, with the exception of South Saskatchewan River, the mean daily maximum temperatures are in the same order, although the differences are only slight. In British Columbia, where the rust persists through the summer, both the mean daily maximum and the mean extreme highest temperatures for the summer months are considerably lower than in the prairie provinces.

The temperature conditions prevailing in the prairie provinces during the summer months and the greenhouse temperatures recorded in Table V are probably not strictly comparable in their effects on host and parasite, as the plants in the greenhouse were grown at two definite levels of temperature whereas in the field the daily temperatures vary from minimum to maximum. Nevertheless it is suggestive that when plants are kept in the greenhouse for 12 hours at a low temperature (slightly higher than the mean daily minimum for July in most districts of the prairie provinces), and for the remaining 12 hours of the day at a high temperature (slightly lower than the mean daily maximum for these provinces), these plants become definitely resistant to stripe rust. The fact that the mean extreme highest summer temperatures (Table VI) rise far above any of the greenhouse temperatures used—the highest was 25.3° C. (77.5° F.)—is an additional reason for supposing that the summer temperatures in the prairie provinces are inhibitory to the growth of the rust during that period. This conclusion is in accord with that arrived at by Sanford and Broadfoot (26) who assume that the stripe rust infestation occurring in Alberta in the autumn months has its origin in wind-borne spores that drift in from the adjoining states of Montana, Idaho, and Washington, rather than in local infections which have survived from the previous year through the severe winter and the hot summer months. Field observations seem to support both of these conclusions, for stripe rust has never been found in Manitoba or the eastern half of Saskatchewan, although it occurs rarely in western Saskatchewan and is present every year in Alberta.

It is conceivable that in an unusually cool summer stripe rust might become more prevalent than it has hitherto been known to be. There is also the possibility that there may exist, or come into existence, a form which is less sensitive to high temperatures than the forms that have been studied in these experiments.

Physiologic Specialization in *Puccinia glumarum*

Physiologic forms of *Puccinia glumarum* were isolated for the first time in Canada in 1932 (20). Their presence in America, however, had been suggested at an earlier date by the work of Hungerford and Owens (14). These

authors found that when *Bromus sterilis* was inoculated with uredospore from *Hordeum jubatum* the former remained resistant, but when it was inoculated with spores from *Bromus marginatus* or *Elymus glaucus* it proved highly susceptible, and they decided that either two forms of *P. glumarum* were involved or that two strains of *Bromus sterilis* had been used. In 1934 Bever (3) was able to show definitely that two forms of *P. glumarum* on wheat, forms 19 and 28*, were present in the United States.

From 1925 to 1928 Rudorf (23) tested a number of wheat varieties to *P. glumarum* in Germany, and found that some of the wheat varieties which Hungerford had classified as resistant to this rust in America were susceptible in Germany. He therefore concluded that the form of *P. glumarum* occurring in Germany was distinct from that found in the United States. In 1930, Allison and Isenbeck (1) demonstrated the presence of four physiologic forms of *P. glumarum* in Europe. About the same time there appeared an article by Gassner and Straib (8) in which they were able to prove the existence of two physiologic forms in west middle Europe, and a few months later Wilhelm (34) reported the existence of five physiologic forms of the organism, three of which he isolated from collections made in Germany, one from France, and one from Sweden.

In Canada systematic annual surveys for physiologic forms of *P. glumarum* were not carried out, but since 1927 collections from wheat, *Aegilops*, barley, and a number of grasses have been studied (Table VII). These collections were made in British Columbia, Alberta, and Saskatchewan. For comparative purposes, two collections of rust from the United States and two from England were studied, together with two from Germany. The separation of the rust cultures into definite physiologic forms was accomplished by means of the wheat varieties selected by Gassner and Straib (9) as differential hosts for *P. glumarum*.

In order to be sure that the reactions of the different hosts were based on race difference and were not the results of environmental influences all tests were made in a greenhouse kept at a temperature of 16° C.

Distribution and Infection Capabilities of the Forms Isolated

The physiologic forms isolated can be seen from Table VII, which gives the hosts upon which the several forms were collected, the year and the place of collection, and a summary of the infection types of each form on all the differential hosts. From this table it is clear that four physiologic forms of *P. glumarum*, forms 4, 6, 8, and 13, were present in the material studied. Of these forms 8 and 13 were isolated from the collections of rust made in Canada; form 6, from Germany; and form 4, from England. Of the two collections from the United States, the one from Berkeley, California, proved to be form 13 and the one from Moscow, Idaho, form 8.

**These numbers do not appear in the paper cited but were supplied recently to the author by Dr. Bever.*

TABLE VII

DISTRIBUTION AND REACTIONS OF PHYSIOLOGIC FORMS OF *Puccinia glumarum*

Form	Place of collection	Year	Host on which collected	Mean reactions on differential varieties										
				Michigan Amber	Blé rouge d'Ecosse	Strubes Dickkopf	Webster C.I. 3780	Holzapfels Früh	Vilmorin 23	Heines Kolben	Carstens V	Spaldings Prolific	Rouge prolifique barbu	Chinese 166
4	Harpenden, England	1931	Wheat	4—	4—	4—	3+	4—	4—	1—	2—	0(n)	0(n)	0(n)
	Newcastle on Tyne, England	1932	Wheat											
6	Halle, Germany	1930	Wheat	4—	4—	4—	3	1+	1—	1—	2	1—	0(n)	0(n)
	Schlanstedt, Germany	1930	Wheat											
8	Duncan, B.C.	1931	*Agropyron trachycaulum* var. *unilaterale*	4—	4—	4—	2—	0(n)	0(n)	0(n)	1—	1—	0(n)	0(n)
	Moscow, Idaho, U.S.A.	1931	*Aegilops cylindrica*											
13	Olds, Alta.	1927	Wheat	4—	0(n)	0(n)	2	0(n)	0(n)	0(n)	0(n)	0(n)	0(n)	4—
	Berkeley, Cal., U.S.A.	1928	*Bromus marginatus*											
	Edmonton, Alta.	1929	*Hordeum jubatum*											
	Victoria, B.C.	1929	*Hordeum jubatum*											
	Olds, Alta.	1930	Wheat											
	Aggasiz, B.C.	1931	*Elymus* sp.											
	Agassiz, B.C.	1931	*Bromus sitchensis*											
	Point Grey, B.C.	1931	*Agropyron trachycaulum* var. *unilaterale*											
	Edmonton, Alta.	1931	*Hordeum jubatum*											
	Edmonton, Alta.	1931	*Agropyron trachycaulum* var. *unilaterale*											
	Edmonton, Alta.	1931	*Agropyron repens*											
	Edmonton, Alta.	1931	*Bromus ciliatus*											
	Olds, Alta.	1931	O.A.C. barley											
	Red Deer, Alta.	1931	*Hordeum jubatum*											
	Saskatoon, Sask.	1931	*Aegilops cylindrica*											
	McLean, Sask.	1932	*Hordeum jubatum*											
	Regina, Sask.	1932	*Hordeum jubatum*											
	Lethbridge, Alta.	1935	*Hordeum jubatum*											

Of the two forms isolated in Canada the less virulent one, form 13, is much more prevalent than form 8, being represented by seventeen of the eighteen Canadian collections studied between 1927 and 1935. Although form 13 has a wider distribution in Canada than form 8, it has not yet been found in Europe.

Resistance of Cereals and Grasses

Resistance of Wheat Varieties

With a view to discovering wheat that might be of value in breeding for resistance to stripe rust, if that became necessary, 52 varieties of wheat were tested to the four physiologic forms. The results of this test are given in Table VIII. In this table the varieties are arranged in groups, each group having something more or less in common with respect to the type of infection produced upon the varieties by the four physiologic forms. In the first group,

TABLE VIII

THE MEAN INFECTION TYPES OF FOUR PHYSIOLOGIC FORMS OF *Puccinia glumarum* ON 52 WHEAT VARIETIES AND HYBRID STRAINS IN THE SEEDLING STAGE

Varieties tested		Origin of physiologic form			
		Germany, Form 4	England, Form 6	Canada	
				Form 8	Form 13
Acme	[1]R.L. 566	4–	4–	4–	4–
Chabot		4–	4–	4–	4–
Chul	R.L. 543	4–	4–	4–	4–
H-44-24	R.L. 229	3+	3+	3+	3
Hope	R.L. 209	3+	3+	3+	3
Hussar	C.I. 4843-1-5	3+	3+	3	3
Kanred × Marquis	R.L. 226	3+	3+	3+	3+
Kota	R.L. 571	3–	3–	3–	3–
Kubanka	R.L. 565	3+	3+	3	3
Malakoff	C.I. 4898-4	3+	3+	3+	3+
Mindum	R.L. 568	3+	3+	3+	3+
Monad	R.L. 205	3+	3+	3+	3
Norka	C.I. 4377-2-1	4–	4–	4–	4–
Parker's	R.L. 71	4–	3+	3+	3–
Pentad	R.L. 203	3+	3+	3–	3–
Prelude	R.L. 25	4–	4–	4–	4–
Reliance	R.L. 198	4–	4–	4–	3+
Reward	R.L. 79	4–	3+	4–	4–
Ruby	R.L. 12	4–	3+	4–	4–
Supreme	R.L. 77	4–	4–	3+	3+
Arnautka	R.L. 570	3+	3+	3+	1+
Axminster	R.L. 75	3+	3+	3	2–
Black Persian	R.L. 388	3	3+	3	2–
Brevit	C.I. 3778-1	3+	3	3+	2+
Democrat	C.I. 3384-3-2	3+	3+	3+	1
Hard Federation	R.L. 921	3+	3+	3+	0(n)
Little Club	R.L. 223	3+	3	3+	2–
Marquis	R.L. 572	4–	3+	3=	2–
Power	R.L. 202	3+	3+	3+	2+
Sevier × Dicklow	R.L. 368	4–	3+	3+	2
Spelmar	R.L. 569	3+	3+	3+	2+
Vernal	R.L. 567	4–	4–	4–	1–

TABLE VIII—*Concluded*

THE MEAN INFECTION TYPES OF FOUR PHYSIOLOGIC FORMS OF *Puccinia glumarum* ON 52 WHEAT VARIETIES AND HYBRID STRAINS IN THE SEEDLING STAGE

Varieties tested		Origin of physiologic form			
		Germany, Form 4	England, Form 6	Canada	
				Form 8	Form 13
Loros	C.I. 3779-4-1	3+	3=	2	2−
N.D. 1656	R.L. 126	3	3	2	2−
Pelissier	R.L. 145	3	3=	1+	1+
Red Fife	R.L. 22	4−	3+	2−	1
Renfrew	R.L. 135	3+	3+	2+	1+
Similis	C.I. 3747-1-1	3	3	1	2−
Svalofs Panzer III		3=	3+	2−	0(n)
Golden Drop		3+	0(n)	0(n)	0(n)
Vilmorins Blé du bon Fermier		4−	0(n)	0(n)	0(n)
Vilmorins Blé gros bleu		3+	1+	0(n)	0(n)
Carina	C.I. 3756-3-5	1−	1+	1	1
Ceres	R.L. 127	2−	2+	2−	2−
Einkorn	R.L. 227	2+	2	2+	2+
Garnet	R.L. 15	0(n)	0(n)	0(n)	0(n)
Iumillo	R.L. 7	1+	1−	1+	1−
Khapli	R.L. 563	2	2	2	0(n)
Marquillo	R.L. 132	1−	1+	1−	0(n)
Mediterranean	C.I. 3384-3-2	2	2+	2	2−
Quality	R.L. 133	1−	1+	0(n)	1−
Rieti		2+	—	2+	0(n)

[1] *Accession numbers of Rust Research Laboratory.*

for example, all the varieties listed are susceptible to the four forms used and bear a "3" or "4" type of infection; in the second group, the varieties are susceptible to three of the four forms; in the third group, they are susceptible to two of the forms; in the fourth group they are susceptible to only one of the four physiologic forms; while in the fifth group, the varieties are resistant to all four physiologic forms. Genetic material bearing the necessary factors for rust resistance is therefore readily accessible to the plant breeder, should such material be required at any time.

Resistance of Barley Varieties

In the early stages of this work it was shown (16) that stripe rust collected on *Hordeum jubatum* was able to cause heavy infections on wheat seedlings in the greenhouse. Later, in 1931, stripe rust collected on O.A.C. 21 barley at Olds, Alberta, caused heavy infections on two of the wheat differential varieties inoculated by it (20). The susceptible reaction of wheat to rust from these sources suggested the possibility that the stripe rust present in Western Canada was capable of parasitizing both wheat and *Hordeum* species. If such were the case there would be reason to doubt the existence of specialized *Tritici* and *Hordei* races of this rust. Accordingly it was decided to test the

reaction of a number of barley varieties to the four available physiologic forms of stripe rust of wheat. The reactions of nine barley varieties to forms 4, 6, 8, and 13 are given in Table IX, from which it is evident that three of the varieties, Glabron, O.A.C. 21, and Success are moderately susceptible to all the four forms.

TABLE IX

THE MEAN TYPES OF INFECTION PRODUCED BY FOUR PHYSIOLOGIC FORMS OF *Puccinia glumarum* ON SEEDLINGS OF NINE BARLEY VARIETIES

Variety tested	Physiologic form			
	4	6	8	13
Bay Brewing	1—	0(n)	0(n)	0(n)
Glabron	3—	3—	3—	3—
O.A.C. 21	3—	3—	3—	3
Peatland	0(n)	0(n)	0(n)	0(n)
Plumage Archer	0(n)	0(n)	0(n)	0(n)
Success	3—	3—	3—	3=
Trebi	0(n)	0(n)	0(n)	0(n)
Velvet	1—	0(n)	1—	0(n)
Wisconsin 38	2—	2—	2—	2—

Resistance of Grasses

In view of the results obtained with barley varieties, it seemed desirable to study the pathogenicity of the same forms towards a number of the native grasses, particularly those of the genera *Agropyron* and *Elymus* which, according to Eriksson (4), harbor the *Agropyri* and *Elymi* races of stripe rust. The results of these tests, which were carried out in the greenhouse with seedling plants, are incorporated in Table X.

TABLE X

REACTIONS OF CERTAIN GRASSES IN THE SEEDLING STAGE TO FOUR PHYSIOLOGIC FORMS OF *Puccinia glumarum*

Grass species tested	Physiologic form			
	4	6	8	13
Agropyron cristatum (Shreb.) Gaertn.	S & R	S & R	S & R	S
A. dasystachyum (Hook.) Vasey	S	S	S	S
A. Griffithsii Scribn. & Sm.	S	S	S	S
A. repens (L.) Beauv.	O(S)	O(S)	O	O
A. repens forma *setiferum* Fern.	O(S)	O(S)	O	O(S)
A. trachycaulum (Link) Malte var. *unilaterale* (Vasey) Malte.	S	S	S	S
A. smithii Rydb.	O(S)	O	O(S)	O
A. trachycaulum (Link) Malte var. *typicum* Fern.	S	S	S	S
Bromus altissimus Pursh.	R	R(S)	R	R
B. ciliatus L.	R(MS)	R	O	R & MS
B. inermis Leyss.	O	R	O	O
B. marginatus Nees.	O	O(S)	R	O(MS)
B. purgans L.	O	R	R	R
B. sitchensis Bong.	R	R	R	R
B. sterilis L.	R	R	R	O
Elymus canadensis L.	S(R)	S & R	S	S
E. curvatus Piper	R(S)	R(S)	O(S)	R(S)
E. dahuricus Turcz.	S	S	S	S
E. glaucus Buckley	R	R	R	R(S)
E. innovatus Beal.	R	R	R	R
E. virginicus L.	S & R	S & R	S & R	R(S)
Hordeum jubatum L.	S	S	S	S
H. murinum L.	O(R)	O	O	O

Explanation of symbols: O = *immune;* R = *resistant;* MS = *moderately susceptible;* S = *susceptible;* S & R = *some plants susceptible; some resistant; brackets* ( ) *indicate that only very few plants showed reaction included.*

Agropyron dasystachyum, *A. Griffithsii*, *A. trachycaulum* var. *unilaterale*, *A. trachycaulum* var. *typicum*, and *A. cristatum* proved rather highly susceptible to all of the four physiologic forms. The remaining *Agropyron* species were chiefly immune but possessed occasional susceptible plants.

Of the *Elymus* species tested, *Elymus dahuricus* and *E. canadensis* showed the greatest degree of susceptibility, the latter, however, exhibiting a small proportion of plants resistant to the two European forms. *Elymus curvatus* and *E. virginicus* were heterogeneous in reaction to each of the four forms, the reactions of individual seedlings varying from resistance to complete susceptibility. *E. glaucus* and *E. innovatus* proved highly resistant.

Of the other grasses tested the *Bromus* species were either immune or rather highly resistant with, however, a few susceptible plants in certain species. *Hordeum jubatum* was completely susceptible to all of the four forms while *Hordeum murinum* appeared to be immune.

The Question of Specialized Forms or Varieties in Stripe Rust

The reactions of barley varieties and grasses to the four physiologic forms mentioned above have an obvious bearing on the question of the specialization of *Puccinia glumarum*. The fact that certain varieties of *Hordeum vulgare* and certain species of *Agropyron* and *Elymus* proved susceptible to the so-called *Tritici* race calls into question Eriksson's (4) division of this rust into the five specialized varieties *Tritici*, *Hordei*, *Secalis*, *Agropyri*, and *Elymi*. It is obvious that if the rust were thus specialized the *Tritici* variety should not be capable of attacking species of *Hordeum*, *Agropyron*, and *Elymus*, even as seedling plants.

Apart from the seedling reactions mentioned above there is available certain other evidence bearing on this question. Sanford and Broadfoot (27) state that *Hordeum jubatum* and *Agropyron dasytachyum* are the principal hosts of *P. glumarum* in Alberta. The present authors have during the past few years established cultures in the greenhouse from 11 collections on *H. jubatum*, 1 collection on *H. vulgare* (O.A.C. 21), 5 collections on *Agropyron trachycaulum* var. *unilaterale*, 1 collection on *Elymus* sp., 1 collection on *Agropyron repens*, and 1 collection on *Agropyron trachycaulum* var. *typicum*. Although the physiologic form present was not in all instances identified, it was demonstrated that every one of the cultures was capable of attacking wheat varieties. Cultures capable of attacking wheat seedlings were also secured from field collections made on *Aegilops cylindrica*, *Bromus ciliatus*, and *Bromus sitchensis*.

In certain cases the physiologic form present was identified (*Vide* Table VII). Thus collections on *Agropyron trachycaulum* var. *unilaterale* and *Aegilops cylindrica* were identified as form 8. Collections on *Agropyron trachycaulum* var. *unilaterale*, *Hordeum jubatum*, *Hordeum vulgare* (O.A.C. 21), *Elymus* sp., *Bromus ciliatus*, *Bromus marginatus*, and *Bromus sitchensis* were identified as form 13.

The fact that the present authors have collected known physiologic forms of wheat stripe rust on species of *Hordeum*, *Elymus*, and *Agropyron*, and have shown that forms 4, 6, 8, and 13 can attack seedling plants of *Hordeum*, *Agropyron*, and *Elymus* species throws a doubt on the existence of the *Hordei*, *Elymi*, and *Agropyri* varieties created by Eriksson.

A review of the literature shows, furthermore, that considerable evidence leading to a similar conclusion has been gathered by other workers. Treboux (32) showed, in 1912, that spores collected on *Agropyron repens* were capable of infecting *Triticum vulgare*, *Hordeum vulgare*, and *Bromus mollis*. Hungerford and Owens (14) in 1923, found that the specialized variety from wheat was able to infect a number of species of *Bromus*, *Agropyron*, *Hordeum*, and *Elymus* as well as rye and to a slight extent barley. Within the last three or four years further confirmation has been secured. Hassebrauk (12) showed that form 4 of *P. glumarum Tritici* infected species of *Secale* and *Agropyron* as well as a species of *Elymus* and concluded that the specialized forms of Eriksson were not so sharply fixed as was supposed formerly. Gassner and Straib (10) collected form 4 of *P. glumarum Tritici* on barley and on *Agropyron repens* and showed furthermore that certain varieties of barley and rye were susceptible, and *Agropyrons repens* at least partially susceptible, to the majority of the known physiologic forms of *P. glumarum Tritici*. On the basis of this work they concluded that Eriksson's division of *P. glumarum* into five specialized varieties was not justifiable. According to a later paper by Straib (31) two of the more recently discovered physiologic forms—forms 23 and 24—attack barley varieties more vigorously than wheat. He does not, however, consider them as representatives of a distinct *Hordei* variety of the rust. He prefers to look upon stripe rust as a species consisting of a series of closely related specialized forms of which these two forms represent the one extreme while the other is represented by physiologic forms which attack wheat varieties more vigorously than barley (30).

Discussion

The limited distribution of *Puccinia glumarum* in Western Canada presents a problem of considerable interest to phytopathologists. Stripe rust is present annually in Alberta on certain grasses and to a slight extent on wheat. Its presence there and its gradual diminution in an easterly direction raise the question of the reason for its limited spread towards the east. The reason is obviously not lack of suitable host plants in the more easterly part of the prairie provinces as the most congenial grass host, *Hordeum jubatum*, is ubiquitous. It is probable, therefore, that the reason is rather to be sought in the climatic conditions prevailing through the growing season. Fraser* and Sanford and Broadfoot (26) have shown that the uredospores are capable, at least occasionally, of surviving the winter in Alberta but, as the last-mentioned authors point out, the further spread of the rust from such survivals is extremely doubtful. They conclude, therefore, that the source of the

* *Unpublished data by Prof. W. P. Fraser, University of Saskatchewan, Saskatoon, Saskatchewan.*

rust infestation which occurs annually in Alberta during the latter part of August and September is to be sought in wind-borne spores from the adjoining States of Montana, Idaho, and Washington.

The extreme sensitiveness of *P. glumarum* in the greenhouse to even moderately high temperatures makes it very probable that the high day temperatures which prevail in the prairie provinces during the summer months prevent the growth of the rust during that period. Greenhouse experiments showed that a temperature of 25° C. (77° F.) for from 10 to 12 hours each day rendered a susceptible host plant resistant to the rust, even when the temperature during the remainder of the day was congenial to both host and parasite. Even if the rust were able to germinate and infect a congenial host under the conditions of temperature that prevail in the summer, it is not improbable that the host plant would be rendered resistant by the high day temperatures which are common in these provinces. It is only in the autumn that stripe rust becomes prevalent in Alberta, the spread coinciding with the shortening of the day and the lowering of the day temperature. These changes in length of day and temperature are, however, so uniform in any given latitude of the prairie region that it is difficult to see how temperature differences could be invoked to explain the limited spread of the rust eastwards. It is probable, as is indeed pointed out by Sanford and Broadfoot (26), that the limited amount of inoculum produced in the stripe rust regions is not sufficient to disseminate the rust very far eastwards before the spread is terminated by the end of the short autumn season.

The behavior of the rust in British Columbia may likewise be interpreted on the basis of temperature conditions. Stripe rust is most abundant during the spring and autumn. The summer temperatures, although lower than those prevailing in the prairie provinces, nevertheless rise above the optimum for rust development, and the rust decreases in abundance during the mid-summer period. With the lower temperatures prevailing in the autumn months the amount of rust again increases. The fluctuation in the amount of stripe rust during these seasons is not likely due solely to temperature conditions. Differences in precipitation undoubtedly play a part. It is probable, however, that the rise in temperature during July and August is one of the chief factors limiting the distribution of stripe rust in that province.

The work reported in the present paper has also a bearing on the question of the specialization of *Puccinia glumarum* which Eriksson (4) subdivided into the five specialized varieties *Tritici*, *Hordei*, *Secalis*, *Agropyri* and *Elymi*. It has been demonstrated that known physiologic forms of wheat stripe rust occur in nature on species of *Hordeum*, *Agropyron*, *Bromus* and *Elymus* and further that forms 4, 6, 8, and 13 are able to attack seedling plants of species of *Hordeum*, *Agropyron*, and *Elymus*. In view of these facts and evidence of a similar nature gathered by other workers in Europe and America, it would seem advisable to disregard Eriksson's division of this rust into five specialized varieties as has, indeed, been suggested by Gassner and Straib (10) and to refer to this rust merely by the binomial *Puccinia glumarum* (Schmidt) Erikss. and Henn.

There can, however, be no question that *P. glumarum* is specialized into a number of physiologic forms which can be detected by the reactions of differential wheat varieties, as was first suggested by Rudorf (23) who found that wheat varieties classified as resistant in the United States were susceptible to stripe rust collected in Germany. The studies reported in the present paper indicate that the physiologic forms prevalent in Canada are different from those commonly present in Europe. All Canadian collections of this rust have been identified as either form 8 or form 13. The former has been found in Europe but the latter, which has not been reported outside of America, appears to be by far the most common form in Canada.

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FRACTIONATION STUDY OF BARLEY AND MALT PROTEINS[1]

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Abstract

The protein of O.A.C. 21 barley and of the malt produced from it were fractionated by means of various solvents and the fractions were analyzed. The results of the solubility studies and of the analyses support the use of 5% potassium sulphate for the quantitative separation of the albumin and globulin from the less soluble proteins. Fractions of the protein undissolved by potassium sulphate solution decreased in amide nitrogen and increased in arginine nitrogen with decreasing solubility. The results suggest that the protein is a complex, only part of which is soluble in 70% alcohol, and that the selection of this solution for separating it into two fractions is entirely arbitrary.

The grains of barley and malt were separated into hulls, germs and "kernels." The total weight and nitrogen content of these fractions, and the distribution of the proteins amongst them was determined. Malting causes no appreciable changes in the hulls. In the "kernel" there is a general breaking down of protein to simpler forms. In the acrospire there is an increase in non-protein nitrogen, salt-soluble protein and glutelin, but no appreciable increase in hordein. The glutelin of the acrospire differs from that of the malt "kernel" both in amide- and argenine-nitrogen content and must be regarded as a distinct protein.

I. Investigation of the Proteins of Whole Barley, Dehulled Barley and Malt

The classification of the cereal proteins is based largely on Osborne's classical work of some thirty years ago (5, 6). Since that time the gluten proteins of wheat have been studied extensively and some of the recent work (4) indicates that gluten is a complex which can be progressively fractionated, rather than a mixture of two distinct proteins as Osborne claimed. By inference, it thus seems possible that the main proteins of barley and malt may also be complexes, rather than mixtures of a prolamine (hordein) and a glutelin. Investigations designed to test this hypothesis are reported in the first part of this paper.

Materials and General Methods

The barley studied was O.A.C. 21, a small-grained, six-rowed, Manchurian variety which is used extensively for malting in Canada. Part of the sample was malted by means of the cage method under the Saladin system. The

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barley and malt contained 2.19% and 2.20% nitrogen and weighed 32.4 and 29.4 gm. per 1,000 kernels, respectively. All samples were ground in a Wiley mill fitted with an 0.5 mm. sieve.

Protein fractions were hydrolyzed in 20% hydrochloric acid for 24 hr. Non-protein compounds were hydrolyzed in 2 *N* sulphuric acid as recommended by Vickery and Pucher (8).

Total nitrogen was determined by the Kjeldahl method. Amide nitrogen was determined by aerating for 2 hr. at 45° C. after titrating to phenolphthalein with sodium hydroxide and saturating with magnesium oxide, and the residue from this determination was used for amide-nitrogen analyses according to the method of Plimmer and Rosedale (7).

Studies with Salt and Alcohol Solutions

Salt-soluble proteins of all cereals are commonly determined by extracting with 5% potassium sulphate, but Blish (2) has pointed out that the selection of this solution is quite arbitrary. The results of some comparisons of the action on barley proteins of this solution and those of other salts commonly in use therefore appear to be worth reporting.

Separate portions of whole barley meal were subjected to five successive extractions of 15 min. each, at room temperature, with chloroform water followed by 5% potassium sulphate, chloroform water followed by 5% magnesium chloride, and chloroform water followed by 8% potassium iodide, and in each case the residue was extracted three times, for 30 min. each, with 70% alcohol in a sealed tube at 82° C. The percentage of the total nitrogen extracted by these methods was determined and the results are presented in Table I.

TABLE I

SOLUBILITY OF PROTEINS OF WHOLE BARLEY MEAL

Initial solvent	Nitrogen extracted by initial solvent, % of T.N.	Nitrogen subsequently extracted by hot 70% alcohol, % of T.N.	Total nitrogen extracted, % of T.N.
Water	19.0	36.2	55.2
5% K_2SO_4	31.6	36.4	68.0
5% $MgCl_2$	36.7	31.2	67.9
8% KI	41.8	27.0	68.8

The data show that although 5% potassium sulphate extracted 12.6% more nitrogen than did water, hot alcohol extracted an additional 36% in each case. The protein extracted by potassium sulphate therefore appears to be insoluble in alcohol. The magnesium chloride and potassium iodide solutions extracted more nitrogen than potassium sulphate but the total nitrogen extracted by the salt and alcohol solutions was not substantially increased. It appears therefore, that 5% potassium sulphate extracts a distinct fraction, whereas the other salt solutions cut across two fractions properly differentiated as salt-soluble and alcohol-soluble.

A number of fractions of barley and malt proteins were prepared and analyzed for amide and arginine nitrogen. Duplicate amounts of whole

meal were extracted successively with water, 5% potassium sulphate and hot 70% alcohol. A second pair of samples was extracted successively with water, 5% potassium sulphate, 30%, 50% and hot 70% alcohol. Owing presumably to more serious denaturation, these successive extractions with increasing concentrations of alcohol did not extract as much protein as one treatment with hot alcohol, and in order to obtain the fraction which was least soluble in alcohol, two more samples were extracted successively with 5% potassium sulphate, 60% alcohol and 70% hot alcohol.

Water extracts were treated with sufficient trichloracetic acid to bring them to 2.5% in respect to this reagent, and after standing over night the protein precipitate was separated by centrifuging. Salt-soluble protein was precipitated by adding sufficient sodium tungstate to give a 1% solution and acidifying with sulphuric acid after the manner of Hiller and van Slyke (3). The alcohol extracts were acidified with hydrochloric acid and boiled to remove the alcohol and concentrate the solution. Nitrogen determinations were made by the methods previously described.

The analytical results are reported in Table II. They show that distinct differences in amide- and argenine-nitrogen content exist between albumin, globulin, hordein and glutelin. When hordein is progressively fractionated, however, it is shown that, with the exception of the most soluble protein

TABLE II

AMIDE AND ARGININE NITROGEN CONTENT OF WHOLE BARLEY AND MALT PROTEIN FRACTIONS

Fraction	Barley			Malt		
	Nitrogen range of fraction, % of T.N. of meal	Amide N, % of T.N. of fraction	Arginine N, % of T.N. of fraction	Nitrogen range of fraction, % of T.N. of meal	Amide N, % of T.N. of fraction	Arginine N, % of T.N. of fraction
Non-protein	0.0 – 9.0	11.2	15.0	0.0 – 21.4	10.6	16.2
Water-soluble protein	9.0 – 19.0	9.2	18.5	21.4 – 37.2	8.7	17.2
5% K_2SO_4-soluble protein	19.0 – 31.6	9.1	22.0	37.2 – 50.0	8.5	21.6
Hot 70% alcohol-soluble protein	31.6 – 68.0	21.0	9.4	50.0 – 74.8	20.6	9.1
Residue	68.0–100.0	10.0	13.6	74.8–100.0	8.0	13.8
Alcohol-soluble fractions						
30% alcohol after H_2O and K_2SO_4	31.6 – 36.8	18.7	11.0	50.0 – 53.2	18.4	14.0
50% alcohol after H_2O, K_2SO_4 and 30% alcohol	36.8 – 50.8	23.5	6.8	53.2 – 61.4	23.5	8.2
Hot 70% alcohol after H_2O, K_2SO_4, 30% and 50% alcohol	50.8 – 65.0	20.2	9.0	61.4 – 69.2	19.6	10.2
Hot 70% alcohol after H_2O, K_2SO_4 and 60% alcohol	59.3 – 67.8	17.8	11.5	68.2 – 72.4	15.6	12.3

which is probably contaminated with small amounts of salt-soluble protein, the fractions decrease in amide nitrogen content and increase in argenine nitrogen content with decreasing solubility, thus approaching the composition of glutelin. If glutelin could be fractionated for analysis it seems possible that it would be found that the fractions increase in amide nitrogen and decrease in argenine nitrogen with increasing solubility and thus approach the composition of the least soluble fraction of hordein. Attempts to demonstrate this experimentally, reported below, were unsuccessful since relatively unchanged fractions of glutelin could not be prepared. Nevertheless, although the evidence is by no means complete, there are some grounds for believing that the main protein of barley or malt is a complex and that the division of it into two fractions by means of hot 70% alcohol is purely arbitrary.

Studies with Acid and Alkaline Solutions

A study was made of the effect on glutelin of aqueous and alcoholic solutions of hydrochloric acid and sodium hydroxide, by methods of extraction similar to those reported above. Alcoholic solutions were more efficient and were more easily employed because they did not swell the starch. At room temperature 0.05 *N* sodium hydroxide in 70% alcohol dispersed about one third of the glutelin and 0.1 *N* and 0.2 *N* solutions dispersed little more. By raising the temperature to 82° C., almost complete dispersion was obtained. Hydrochloric acid was not as effective. At 82° C. the extraction curve was almost identical with that obtained with sodium hydroxide at room temperature. A 0.1 *N* solution of acetic acid dispersed very little of the glutelin fraction even at 82° C. It was readily demonstrated, as could have been predicted, that with these concentrations of reagents and particularly at the higher temperature, amide nitrogen was hydrolyzed from the protein, and suitable fractions of glutelin for analysis therefore could not be prepared by means of these reagents. It is worth noting, however, that just as the hordein-glutelin complex can be divided at any point in the hordein range by choosing the right concentration of alcohol and the right temperature, so can it be divided at any point in the glutelin range by choosing a suitable temperature and concentration of sodium hydroxide in 70% alcohol.

Studies with Sodium Salicylate Solutions

Attempts were made to fractionate the proteins of barley and malt by means of sodium salicylate solutions. It appeared that the use of this solution, which has been used effectively in studying wheat proteins, might serve not only to produce suitable fractions of the least soluble proteins for analysis but also to provide further evidence respecting the solubility of the other proteins. Barley proved to be far less amenable than wheat: comparatively pure protein, corresponding to washed wheat gluten, cannot be obtained, and studies of the whole meal were made difficult by the low concentration of the protein and the interfering action of the starch, which swells in concentrated solutions of sodium salicylate.

Since it was not possible to produce by means of this reagent suitable progressive fractions of hordein and glutelin for analysis, the investigations as a whole proved fruitless. Nevertheless, a selection of the solubility studies appears to merit brief description.

Samples of whole barley meal of such size as to contain 50.0 mg. of nitrogen were extracted at room temperature on a mechanical shaker, first with 50 ml. of solution for 3.5 hr. and then with three 25 ml. portions for 0.5 hr. each. The extractions were carried out with various concentrations of sodium salicylate solution, and the percentage of the total nitrogen extracted by each concentration was determined. The process was also repeated with whole-malt meal and meal prepared from barley from which the hulls and germs had been removed by means of a wire-brush mill. The results are shown in Fig. 1 in the form of a graph.

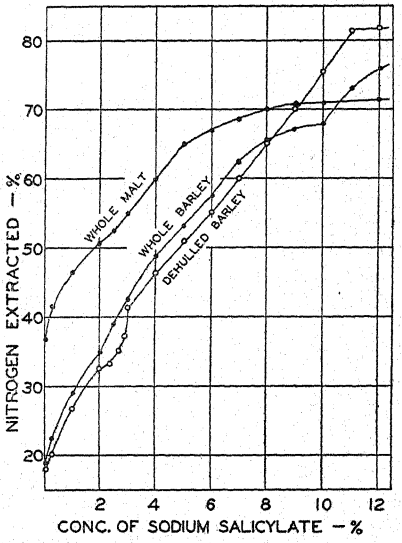

FIG. 1. *Extraction of barley and malt with sodium salicylate solutions.*

The definite irregularity in the curve for dehulled barley, and the slight irregularity in that for malt, beginning at about 2% sodium salicylate indicate differences in the solubility of the protein extracted before and after this point. Since this concentration of sodium salicylate extracts approximately the same percentage of nitrogen from the respective products as 5% potassium sulphate, these results support the earlier conclusion that the latter reagent serves to divide the proteins at a logical point.

No particular importance is attached to the irregularity in the curve for whole barley at 10% sodium salicylate. It was noted that this solution swelled the starch but did not disperse it, whereas a 12% solution dispersed it to such an extent that it had to be decanted with the extract and in all probability it carried some undispersed protein with it and thus produced the irregularity in the curve. In experiments with dehulled barley the amount of starch decanted with the extracts appeared to be proportional to the concentration of sodium salicylate, and thus no irregularity was created.

II. The Distribution of Proteins Amongst the Physical Fractions of Barley and Malt

Current methods of fractionating barley and malt proteins, in which the whole of the kernel is ground up before extracting with various solvents, take no cognizance of differences in the distribution of the protein in various parts of the kernel or of possible qualitative differences in proteins of the

same solubility occurring in different parts of the kernel. The literature on malting appears to contain no useful body of data on this subject, and because of its importance in elucidating the changes which take place during malting, and of its bearing on the validity of the methods now used in studying barley and malt proteins, preliminary investigation of it was undertaken.

Materials and Methods

The materials and the methods used for extraction, hydrolysis and nitrogen determinations were identical with those described previously.

The separation of the physical fractions of the whole grains was carried out as follows:—The hulls and embryos were separated from the "kernels" by means of a wire-brush mill. The "kernels," hulls and germs of barley were then separated by sifting and fanning. It was necessary to resort to hand picking in order to separate the malt acrospires from small pieces of "kernel."

Total Weight and Nitrogen Content

The results of the determinations of the total weight and nitrogen content of the various physical fractions of barley and malt are reported in Table III. The hulls of both barley and malt, though forming a considerable portion of

TABLE III

TOTAL WEIGHT AND NITROGEN CONTENT OF PHYSICAL FRACTIONS OF BARLEY AND MALT
(All figures reported on dry basis)

Physical fraction	Weight		Nitrogen		
	gm. per 1000 kernels	% of whole grain	% of fraction	mg. per 1000 kernels	% of T.N. of whole grain
Whole barley	32.4	100	2.19	710	100
Hulls	3.9	12	0.40	16	2.2
Germs	0.63	2	6.30	40	5.6
Kernels*	27.9	86	2.34	654	92.2
Whole malt	29.8	100	2.20	656	100
Hulls	3.9	13	0.41	16	2.4
Acrospires	1.4	5	5.32	75	11.4
Kernels*	24.5	82	2.31	565	86.2

**Whole grain less hulls and germs. Values obtained by difference.*

the whole grain, are low in nitrogen and thus contain only a small proportion of the total nitrogen. The germs, on the other hand, are high in nitrogen and thus, particularly in malt, contain a very appreciable proportion of the total nitrogen. For these reasons alone, studies made with the whole grain must fail to present a clear picture of the changes which take place during malting in the most important part of the kernel, that is, the endosperm.

Distribution of the Proteins

The distribution of the proteins amongst the physical fractions of barley and malt, as shown by determinations of the total nitrogen of protein fractions extracted by the classical solvents, is shown in Table IV.

TABLE IV

DISTRIBUTION OF BARLEY AND MALT PROTEINS

Nitrogen fraction	Barley				Malt			
	Kernels*	Hulls	Germs	Whole barley	Kernels*	Hulls	Germs	Whole malt
Nitrogen in mg. per 1000 kernels								
Non-protein	53	5	6	64	117	7	16	140
Water-soluble	58	2	11	71	91	1	11	103
5% K_2SO_4-soluble	87	0	3	90	81	0	3	84
Hot 70% alcohol-soluble	253	1	3	257	158	1	4	163
Residue	203	8	17	228	118	7	41	166
Total	654	16	40	710	565	16	75	656
Nitrogen as % of total nitrogen of fraction								
Non-protein	8	31	16	9	21	43	22	21
Water-soluble	9	13	28	10	16	7	15	16
5% K_2SO_4-soluble	13	0	7	13	14	2	4	13
Hot 70% alcohol-soluble	39	6	6	36	28	9	5	25
Residue	31	50	43	32	21	39	54	25

**Whole grains less hulls and germs. Values obtained by difference.*

The data show that malting affects different parts of the grain in different ways. Practically no change occurs in the hulls. In the "kernel" there is a general breaking down of protein into simpler and more soluble forms. This results in large increases in the non-protein and water-soluble protein which take place at the expense of the hordein-glutelin fraction. The amount of salt-soluble protein remains almost unchanged. On the other hand, this process is reversed in the germ. It is true that the non-protein nitrogen is increased, but the protein itself is also built up into the least soluble form, the amounts of water-soluble, salt-soluble, and alcohol-soluble protein remaining almost unchanged. The results of the investigation demonstrate that a more accurate picture of the changes which occur during malting can be obtained by separating the grain into its various physical fractions before analysis.

The results of the analyses of whole barley and whole malt agree with those of Bishop (1) except that he found that the salt-soluble protein increased during malting. This difference can probably be accounted for by differences in the barleys and the malting methods used in the two investigations. Bishop found that, during malting, glutelin first decreased and then increased. The increase was attributed to re-synthesis in the embryo and rootlets and the

first part of this hypothesis is supported by the results of the present investigation. It was also suggested that the decrease in the rate of disappearance of hordein might be due to re-synthesis in the embryo. This suggestion is not substantiated by the data presented in Table IV, which show that the acrospires contain little alcohol-soluble protein.

Analyses of Acrospire Proteins

As several grams of acrospire meal were available and as this fraction is high in nitrogen, it was possible to obtain some protein fractions of it for arginine and amide nitrogen analyses. There was so little of the salt-soluble and alcohol-soluble fractions that it was impossible to analyze them, but the results for the other fractions are reported in Table V.

TABLE V

AMIDE AND ARGININE CONTENT OF MALT ACROSPIRE NITROGEN FRACTIONS

Fraction	Amide N, % of T.N. of fraction	Arginine N, % of T.N. of fraction
Non-protein	12.6	22.4
Water-soluble	7.6	21.2
Residue	5.5	24.4

The data are extremely interesting. If they are compared with similar data for the proteins of whole barley and malt given in Table II, it will be observed that the acrospire proteins are entirely different. The residue protein is, in fact, so different in both its argenine- and amide - nitrogen content that it must be regarded as a distinct protein rather than as malt glutelin. In these circumstances it is not surprising that some difference of opinion has existed in the past on whether the proteins extracted from whole barley and malt were identical. It appears that by suitable fractionation it can be demonstrated that they are not identical.

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RHIZOPHIDIUM GRAMINIS N. SP., A PARASITE OF WHEAT ROOTS[1]

BY G. A. LEDINGHAM[2]

Abstract

A Chytrideaceous parasite apparently not hitherto described has been found on the roots of wheat seedlings grown in soil from Ottawa, Ontario; and Boston, Massachusetts. The fungus is closely related to the uniporous Rhizophidiums but since it does not appear to be identical with any of these morphologically and exhibits a type of sexuality not previously described in this genus it is considered to be a new species. The name *Rhizophidium graminis* is proposed.

Introduction

In the past, mycologists have reported a number of species of *Rhizophidium* growing on algae, aquatic fungi, lower animals and pollen grains in water. As far as the writer is aware, no species of this genus has been described as growing on the living roots of higher plants. Hence the present species, parasitic on such roots, is of additional interest; it is moreover apparently a new species, and for these reasons the following notes concerning it are presented in the hope they may prove of interest to other workers in the same field.

Materials and Methods

In 1930, a few sporangia of this *Rhizophidium* growing as a parasite on the roots of wheat were noticed while examination was being made for an intracellular root parasite in the same host. Infection was in general light and very scattered, but at times, and on certain specimens, the fungus was plentiful.

Preliminary studies were made at the University of Toronto in the spring of 1932, with material which was found infecting roots of wheat grown in soil from the Central Experimental Farm, Ottawa. During the fall of the same year further observations were made at the laboratories of Cryptogamic Botany of Harvard University, using material brought from Ottawa, and additional material was provided from naturally infected plants of a species of *Panicum* growing by a small pond in the grounds of the Arnold Arboretum, Boston. When Marquis wheat was grown on soil in which this grass was present, excellent infection was obtained in two to three weeks.

Most of the studies were made on living material in water mounts, but in some cases lacto-phenol was used as a preservative. Mounts stained with cotton blue or acid fuchsin in this medium were particularly useful in demonstrating the extent of the rhizoidal system.

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This study was begun in the Botany Department, University of Toronto, during the tenure of a National Research Council Scholarship and a research assistantship in the Department; it was continued in the Laboratories of Cryptogamic Botany, Harvard University, and completed in the National Research Laboratories, Ottawa. Listed as Contribution No. 128 of the Laboratories of Cryptogamic Botany, Harvard University.

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Diagnosis

As the fungus from these two sources, when investigated intensively, ıroved to be the same species and showed definite points of distinction in ıorphology as well as in host habitat, differentiating it from the species lready reported, there seems to be justification for describing it as new with he following diagnosis:

Rhizophidium graminis sp. nov.

Zoosporangiis extramatricalibus solitariis vel gregariis maturitate 10–75 μ liametro globosis vel ellipsoideis, levibus, basi filamentis myceleis tenuibus praeditis, eisdem in cellula matricis penetrantibus; zoosporis numerosis 3 × 1.8 μ, apice zoosporangeorum fracto erumpentibus, guttula hyalina praeditis atque basi attenuato cilio uniquo; sporangii perdurantibus 6–12 μ liam. globosis, endosporis levibus et exosporis asperis.

Rhizophidium graminis n. sp.

Zoosporangia 10–75 μ in diameter, globose to ellipsoid, with smooth walls, solitary or gregarious, giving rise at the base to fine rhizoids which ramify within the host cell. Zoospores 3 × 1.8 μ uniguttulate and uniciliate, the cilium 10–12 μ in length, liberated in large numbers by the rupturing of the apex of the sporangium. Resting spores 6–12 μ diameter, globose with smooth endospore and rough scabrous exospore wall and oily protoplasmic contents, sexual fusions occurring between rhizoids of two thalli. Germination of the spore takes place by the extrusion of the cell contents to form a thin-walled sporangium.

Parasitic on roots of *Triticum* and *Panicum* spp. in Ontario and Massachusetts.

Specimens have been deposited in the Farlow Herbarium, Harvard University and the Mycological Herbarium, Department of Botany, University of Toronto.

Observations on the Morphology of the Fungus

The Zoosporangial Phase

This phase is initiated when the amoeboid zoospore attaches itself to the root hair or to the epidermal cell of the wheat root and begins to develop directly into the zoosporangium. The youngest stage found, about 4 μ in diameter, contains a fine-grained protoplasm in which are embedded numerous larger particles and several vacuoles (Fig. 1).

The rhizoidal system, which supplies the growing thallus with food from the host, develops as a small outgrowth which arises from the side of the zoospore in contact with the host cell. After the primary rhizoid has penetrated the host-cell wall, growth continues, the rhizoids at first being rather short, thick and unbranched, with blunt terminals. But later, owing to their profuse branching they develop into a densely woven mass of very fine threads (Fig. 2). There is no evidence of a subsporangial vesicle or any enlargement at the base of the zoosporangium such as one finds in the genus *Phlyctochytrium*.

Meanwhile growth of the sporangium has been taking place simultaneously with the development of the rhizoids. The vacuoles and coarse granules characteristic of early stages have disappeared and the content has become homogeneous (Fig. 2). Numerous refringent granules next appear, making it very difficult to follow the process of zoospore delimitation. Ultimately the mature sporangium is closely packed with these oil-like droplets, one of which is included in each zoospore. During the process of zoospore formation there is very little movement of the contents of the sporangium, and even after the zoospores are fully formed they remain motionless, with no suggestion as to when discharge is about to take place. The sporangia show great variations in size at maturity, measuring as little as 10 μ and as much as 100 μ in diameter and containing, according to their size, from 8 to more than 100 zoospores.

Zoospore discharge was controlled most successfully by varying the amount of soil moisture. If the soil was allowed to become slightly dry, zoosporangia developed and matured normally but active zoospore discharge did not take place. In material kept under these conditions for several hours there was an accumulation of ripe sporangia which, when placed in water, released zoospores almost immediately.

Zoospore discharge is of a characteristic type in this species in that the wall of the sporangium bursts suddenly, forcibly ejecting the zoospores. These remain in a quivering mass for a moment, then swim away in all directions. The empty cup-shaped basal wall of the sporangium with its irregularly torn upper margin persists on the host-cell wall for several days (Fig. 6). Occasionally the tear may proceed regularly, leaving a cap-like structure (Fig. 5). The persistent nature of this wall is in contrast to the evanescent character of the same in *R. clinopus*, which according to Scherffel (4) lasts only a few hours.

It is quite possible to distinguish *Rhizophidium graminis* from other species of the genus by this characteristic method of zoospore discharge. In other species discharge takes place through pores, as in *R. pollinis*, or by exit tubes as in *R. transversum*, or by gelatinization of the tip of the sporangium as in *R. clinopus*. There is no indication in *R. graminis* that the zoospores are surrounded by a vesicle or embedded in "slime" as are those of *R. sphaerocarpum* described by Sparrow (7, p. 519), when they are freed from the sporangium.

Active zoospores are usually pyriform in shape and average 3 by 1.8 μ in size. At the blunt anterior end lies the conspicuous refringent granule referred to previously. The fact that this drop blackens in osmic acid, dissolves in ether and reacts to fat stains, indicates that it is of an oily nature.

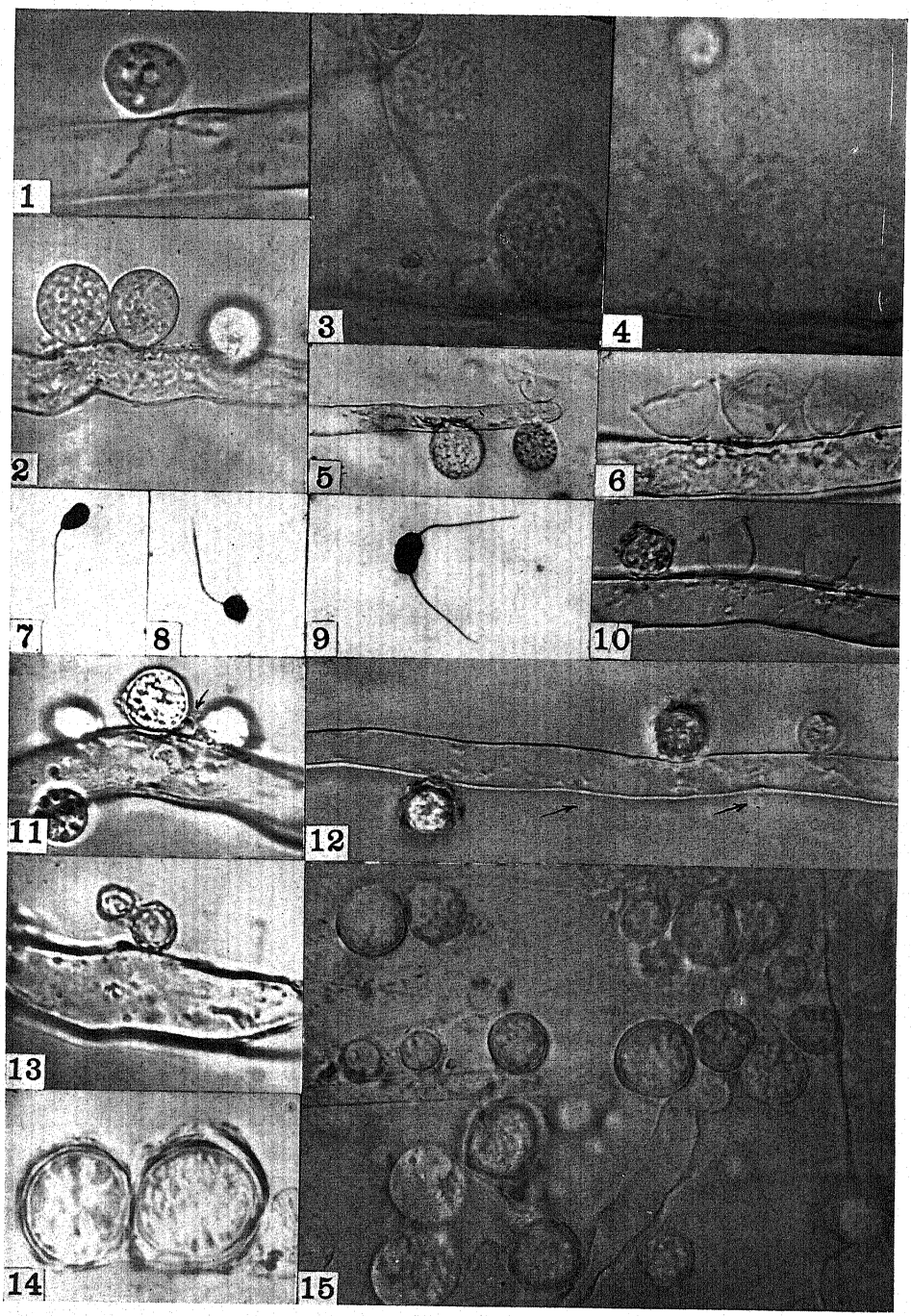
Zoospores may be readily stained by using Cotner's method (1). They usually become nearly spherical when killed in osmic acid fumes (Fig. 8), but some retain their original form (Fig. 7). Near the anterior end of the spore occur several coarse granules which stain heavily. The nucleus lies near the posterior end, and close by is the blepharoplast from which arises the long, straight conspicuous cilium, 10 to 12 μ in length.

On many occasions, in stained preparations and also in hanging drop mounts, biciliate swarmers twice the usual size were found. They were conspicuous because of their awkward rolling motion when swimming. As biciliate zoospores were never seen at the time of zoospore emission, or for some time after, it would seem that they must arise as a result of fusion between two zoospores, rather than from incomplete cleavage. Observations on the process of fusion in hanging drop mounts supports this view. Fusion takes place in the same manner as described by Kusano for zoospores of *Synchytrium fulgens* (3). Prior to fusion the zoospores creep about sluggishly and send out pseudopodia. Sometimes two touch but break apart again. When first joined, only a small part of the surface of one is in contact with the other. The two cilia are at opposite poles, two refringent granules are present, and the line of fusion is marked by a deep constriction. This constriction gradually disappears, the oil globules fuse and the spore becomes ovoid in shape, measuring 4.5 by 2.5 μ (Fig. 9).

The Sexual Fusion

Zoosporangia of *R. graminis* are produced by the enlargement of a single zoospore which has developed rhizoids within the host cell. No sexual mechanism is concerned in the production of this phase, nor indeed is it to be expected. In the resting spore which appears somewhat later it seemed logical, judging from the course of events in many related organisms, to expect a fusion somewhere during development. When an occasional fusion between two zoospores was first found it was thought that perhaps the resulting biciliate spore was a zygote, which after infection would give rise to a resting spore. However, when a small, undeveloped thallus, almost devoid of contents, was found attached to the host near an immature resting spore, doubt arose as to whether this was the correct interpretation to put on such fusions. By careful searching near many resting spores it was found that this empty cell was generally present. Sometimes it lay against the resting spore close to the base (Fig. 11), but it always remained an independent body, not connected in any way as is the companion cell found in *Rhizophidium globosum* (2). The contents, therefore, could not have passed from one to the other except through the rhizoids, which were closely intermingled. It was found that even where the two thalli were some distance apart the rhizoids were in contact with one another (Fig. 12). Undoubtedly transfer of nuclear material from one to the other had been accomplished through the rhizoids. The empty cell might, therefore, be considered a male gamete, which had fertilized the female now developing into a resting spore.

Petersen (4) described a type of fusion similar to this in *Siphonaria variabilis* in 1903. Dr. F. K. Sparrow who has recently observed the same phenomenon in *Rhizoclosmatium* and verified Petersen's observations on *Siphonaria*, kindly examined some material of *Rhizophidium graminis* and called the attention of the writer to the similarity.



Rhizophidium graminis. All figures except 7, 8 and 9 from living material. FIG. 1. *Young zoosporangium. ×1300.* FIG. 2. *Zoosporangia prior to segmentation into zoospores. ×455.* FIG. 3. *Mature zoosporangia. ×790.* FIG. 4. *Same as Fig. 3. a few minutes later. ×790.* FIG. 5. *Empty zoosporangium and two others beginning to form zoospores. ×455.* FIG. 6. *Remnants of zoosporangia after zoospore discharge. ×1300.* FIG. 7. *Stained zoospore. ×1300.* FIG. 8. *Stained zoospore. ×1300.* FIG. 9. *Stained biciliate zoospore formed as a result of fusion of two uniciliate spores. ×1300.* FIG. 10. *Resting spore and remnants of two zoosporangia. ×1300.* FIG. 11. *Resting spore with male cell, indicated by arrow, lying close to it. ×850.* FIG. 12. *Resting spores, with male cells, indicated*

The Resting Spore

The mature resting spores vary from 6 to 12 μ in diameter, and there i some correlation in size and, indeed, in shape, with the location on the host for the smaller spores are generally developed on root hairs and occur singly and scattered, so that they attain a spherical shape, whereas the larger one develop on the cells of the epidermis, and usually occur in groups or in rows so closely packed that their sides are flattened.

In the dense protoplasm of these resting spores numerous refringent granules are present (Figs. 12, 14 and 15) the coarseness of which distinguishes them from the finer granules characteristic of the zoosporangium (Figs. 2, 3 and 5), but there is no large central oil globule such as is found in the resting spores of *Rhizophidium pollinis* (2).

Two walls are present, a smooth hyaline inner one about 0.5 μ in thickness, formed at an early stage in the development of the spore and an outer one, developed later, generally irregularly rough and scabrous (Figs. 12 and 13), but in some old spores in disintegrating root material, appearing almost smooth (Fig. 15).

The complete process of germination has not been observed. However, when spores which had been frozen in ice for several weeks were returned to conditions favorable for growth, the content in one instance was extruded into a thin zoosporangium-like sac, which increased in size for several hours, until it had attained dimensions almost equalling those of the resting spore itself, as shown in Fig. 13. Even though the formation of zoospores in this extruded material did not take place it seems logical to assume that this represented the preliminary stages of germination, for the process was very similar to that described by Sparrow for *Chytridium schenkii* (6).

Conclusion

Several attempts have been made to cultivate *R. graminis* on various culture media but without success. Until some method is obtained of growing the fungus in pure culture it will be difficult to test its pathogenic effects on the host. The present observations, though perhaps not too adequately worked out, are presented in the hope that they will be of interest to others studying the root parasites of plants.

Acknowledgments

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STUDIES ON DROUGHT RESISTANCE IN SPRING WHEAT[1]

By O. S. Aamodt[2] and W. H. Johnston[3]

Abstract

The results of a number of preliminary investigations regarding the nature of drought resistance in wheat are presented.

A study of the susceptibility of wheat plants to drought at different stages of development showed that the shooting and the soft-dough periods were the most critical, from the point of view of grain yield. Heavy foliage losses occurred when plants were exposed during the shooting period, and recovery was very slow. Exposure of plants during the stooling stage resulted generally in transient injury, as the capacity for recovery was particularly great at this time. Plants subjected to drought during the hard-dough stage sacrificed little in kernel plumpness.

Hardening of wheat plants by soil drought, or by limited exposures to atmospheric drought, increased their resistance to exposures of severe atmospheric drought.

The drought-resistant varieties Milturum and Caesium were found to possess a more highly branched primary root system than the non-resistant varieties Marquis and Reward. The durum varieties, Pentad and Pelissier, were found to excel in numbers of primary roots produced, having an average of five per plant. Milturum, Caesium and Baart had more than four primary roots per plant on the average; while Canus, Federation, Reward, Marquis, Garnet and Red Bobs No. 222 had from 3.2 to 3.6. Reliance wheat and wild oats had not more than three primary roots per plant.

The addition of superphosphate to the soil resulted in a slight decrease in number of primary roots of Milturum, Caesium and Marquis, and a slight increase in the number of Reward.

The kernels of the drought-resistant varieties, Milturum and Caesium, did not show any marked superiority to those of Marquis, Reward, Garnet and Red Bobs No. 222, in capacity to germinate in the more concentrated solutions of sodium chloride, potassium chloride and sucrose. A higher energy of germination was displayed by the kernels of Milturum, Caesium and Garnet than by those of the other three varieties tested.

Milturum and Canus exhibited high resistance to injury from windburn when tested in the late stooling to early shooting growth stages. The durum varieties Pentad and Kubanka and the soft wheat, Baart, on the other hand, showed high susceptibility. The soft wheat, Federation, and the variety Red Bobs No. 222 were moderately susceptible.

Introduction

Drought is probably the major limiting factor in crop production in the semi-arid regions of the world. Within these regions are found the principal wheat-producing areas of Canada, United States, Argentine, Australia and Russia. Periodically these semi-arid areas are subjected to conditions of drought, which may be so severe as to cause a virtual crop failure. The disastrous harvest of 1921 in Russia due directly to drought conditions caused a national calamity, and destroyed by famine and accompanying disease millions of human lives (35). The same country suffered a drought of even greater intensity in 1892 (35). There is seldom a year in which some part of

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the wheat-growing areas of the world is not subjected to drought of varying intensity. It has been estimated that the total yield of wheat on the Canadian prairies during the five years, 1930–34, has been reduced on the average by approximately 75,000,000 bushels. This loss is equal to that resulting from black stem rust in epidemic years.

Breeding for drought resistance in cereals has received little attention from investigators, except in Russia, where it received great impetus after the drought of 1921 (35). In Russia, the nature of drought resistance and its application to breeding problems are being studied. Already several new drought-resistant wheat varieties, superior in yield and quality to the older Russian varieties, have been developed by the plant breeders of that country (41, 43).

It is only comparatively recently that research projects have been developed in America to combat drought. In 1928 the University of Alberta received a number of Russian wheat varieties that had been developed for areas of low rainfall. A number of these proved to be superior to local varieties in resistance to drought, but were inferior in certain agronomic characters, as well as in quality. In 1929 a breeding investigation of drought resistance was started when crosses were made between the drought-resistant Russian varieties and the improved local varieties. In 1931, financial assistance was received from the National Research Council of Canada, and a comprehensive programme for the study of drought resistance was begun. The following fourfold programme is being followed:

(a) To ascertain the resistance of wheat varieties to drought.

(b) To study in detail certain morphologic and physiologic characters associated with drought resistance.

(c) To study the mode of inheritance and genetics of drought resistance.

(d) To produce desirable strains of cereals resistant to drought.

The results discussed in this paper pertain to preliminary investigations conducted regarding sections *(a)* and *(b)* of the programme outlined above.

The results of certain investigations conducted on the *(c)* and *(d)* phases of the problem have been summarized by Aamodt and Torrie (4).

Literature Review

Although considerable work has been conducted on the physiology of drought resistance in plants, little has been accomplished in the way of breeding resistant varieties. A possible explanation for this state of affairs is the failure of investigators to recognize the complex nature of drought resistance. The bulk of the work reported represents attempts to find some simple index of measuring drought resistance by means of a number of relatively easily observable anatomical or physiological characteristics. The more modern school of thought, as represented by Maximov (26), believes that the only true criterion of drought resistance is the ability to endure drought with-

out injury. Drought resistance possessed by any group of plants is due to a multiplicity of morphological and physiological characteristics, which investigators have, so far, failed to separate into their component parts.

The difficulty of obtaining a true conception of what is meant by drought resistance is complicated at the outset by the existence of two types of drought, each of which presents to the investigator a distinct series of problems. The type of drought that is best known, and which has received the greater amount of attention in the past, is that occurring when, under normal atmospheric conditions or otherwise, the soil ceases to provide the plant with sufficient moisture to take the place of that lost by transpiration. Drought of this nature is commonly known as soil or edaphic drought. The second type of drought, which appears to have gained recognition only relatively recently, is known as atmospheric drought, and is usually caused by hot dry winds, which may produce desiccation of the plant, even under conditions of relatively high soil moisture. Shantz (39) in his work on water requirements of plants gave special attention to the soil type of drought. Vasiliev (48) has stated that there appears to be no ground for believing that it is possible to breed plants resistant to soil drought, while the breeding of plants resistant to atmospheric drought should be possible.

Drought resistance has been found to be a variable factor and depends to a large extent upon environmental conditions prevailing before drought sets in. Tumanov (44) found that repeated wilting of sunflowers resulted in a "hardening" process analogous to that observed at low temperatures (17, 30, 31). Kondo (23) observed the same phenomenon to occur in the case of soybeans as well as in sunflowers. Kondo also found that plants grown under conditions of insufficient soil moisture, suffered considerably less injury from exposure to severe conditions of soil drought than plants grown under conditions of optimum soil moisture. Tumanov (45) noted that oat plants grown in the shade were less resistant to soil drought than plants grown in direct sunlight.

Even the different organs of the same plant have been found to vary in their degree of drought resistance. Bartholomew (7) found that the suction tension developed in the leaves of wilting lemon plants withdrew water from the fruit, causing a pathological disturbance known as "internal decline of lemons". Lloyd (25) noted that a similar phenomenon caused the dropping of young buds of cotton plants. Tumanov (45) found that leaves of sorghum withdrew water from the stem under stress of soil drought, while the leaves of buckwheat lacked this ability. This investigator also found that the aerial parts of the alfalfa plant exhibited less drought resistance than did the crown and the roots. Krasnosselsky-Maximov (24) cites instances in which the leaves of cereals drew water from the inflorescences when under exposure to artificial dry winds. She also observed that when the leaves were removed before exposure of the plants to this wind, the inflorescence had a much higher water content at the end of the exposure than inflorescences of plants with the leaves present.

It is also well known that plants exhibit different capacities for enduring drought conditions at various stages of their development. The mature seed can undergo almost complete desiccation, without apparent harm. This resistance appears to be manifested also for the first few days following germination. Beal, cited by Robbins (38), germinated seeds of wheat and buckwheat six times, each time allowing the root and stems to grow to the length of the grain before stopping the process by dryness. He found that five repeated germinations were necessary before the percentage germination of these seeds was materially reduced. With the development of leaves, however, the plants became susceptible to severe injury by desiccation. Brounov (26) found that cereals were most susceptible to drought during the period of rapid growth of the culm prior to heading. He applied the term "critical period" to the stage in which the plant was most susceptible. Moliboga (29), Pullman (26), Azzi (6) and Beauverie (8) have also studied the critical periods in cereals and have confirmed the findings of Brounov.

To the plant breeder the study of critical periods in their relation to peculiarities of local climate is of utmost importance. Only those varieties should be selected for a given area, whose critical periods do not coincide with the greatest combination of adverse meteorological factors. Ivanov (20) cites the example of Turkestan wheat, which develops very rapidly during the early stages of growth and comes into head long before other types. The second stage is slow, thus enabling the plant to withstand any early drought, and utilize late rains. Talanov (43) found that the variety Milturum 0.321, owing to its long stooling period (a relatively drought-resistant stage), was able to withstand early summer drought and avail itself of late rains and more suitable atmospheric conditions.

These examples serve to show that it is possible for plants, by means of a delayed or an advanced critical period of growth, to escape regularly recurrent periods of drought. It is important to remember that such plants cannot be considered as truly drough-resistant, since they would succumb in the event of an off-season drought. However, they are of great importance from an agricultural point of view in areas subjected to regular seasonal droughts.

Many phases of the drought resistance problem have been investigated with the hope of obtaining some relatively simple and practicable index for determining drought resistance. The relation of the root system to drought resistance has been the subject of considerable research. Earlier investigators in this field, Gain (16) and Hedgecock (18) believed that the relative drought resistance of plants might be determined by the degree to which they availed themselves of the soil moisture. This earlier belief was modified by Briggs and Shantz (10), who showed that different species of plants when grown on the same soil type showed little, if any, difference in "wilting coefficient". They did find that different soils varied considerably in this regard. Caldwell (13) noted further that the wilting coefficient varied with environmental conditions. Plants growing under humid or shady conditions could reduce the water content of the soil to a much greater degree than those grown under

dry or sunny conditions. Ivanov (20) has stressed the importance of the root system in relation to drought resistance. He points out that plants with roots of high suction power are in the most favorable position to resist drought. He believes the denseness of the root hairs to be an important characteristic of a resistant plant. It enables the plant to tap a much greater volume of soil than it would otherwise. He believes, furthermore, that these factors are important under conditions of high transpiration (atmospheric drought), when a rapid moisture supply is necessary to prevent wilting of the plant.

Talanov (43) reported that the drought resistance of Caesium and Milturum, two West Siberian wheats, was due in part to both a rapidly developed primary root system, and a profusely branched root system in its ultimate aspect. He states that "the root systems of these varieties touch the soil particles with a greater surface and absorb water from a greater volume of soil (up to the limiting minimum, connected with the peculiarities of the soil)." Miller (28) reported that the nature of the root system may play an important role in preventing incipient wilting—the drying out of the epidermal cells. The rate of transpiration of sorghum per unit of leaf area was found to be twice that of corn, while the leaf area was only 25% as great. An examination of the root systems of these two crops showed that, while both had the same number of roots of the first order, sorghum possessed twice as many roots of the second order. Miller believes that the greater number of finer roots of sorghum, together with the smaller leaf surface, are instrumental in keeping the water supply of the leaf sufficient to retard incipient wilting.

For a long time xerophytes were believed to have low transpiration rates. Maximov and his associates (26) have shown that, contrary to the former belief, xerophytes were characterized by high transpiration rates. Zalenski (26), Ivanov (20), and Wilson (49) have corroborated these findings.

Briggs and Shantz (11, 12) and Shantz and Piemeisel (40) were unable to confirm their original premises of a close association between the water requirement of plants and drought resistance. They found that millet, sorghums and corn have a lower water requirement than wheat, oats or barley. The drought-resistant grasses, Agropyrons and bromes, were found to have a very high water requirement; while rice and buckwheat, relatively drought-susceptible species, showed a moderately low water requirement. Maximov and Alexander (26) were also unable to show a direct relation between water requirement and suitability for arid areas. They did find that there was a tendency for plants that transpired intensively to use the water less efficiently. Tulaikov (26) concluded that the water requirement of different members of the same species was quite similar. The differences were too small to justify making selections on this basis. Kiesselbach (21) came to similar conclusions in regard to the value of water requirement in selecting drought resistant strains of corn. Richardson (36), working in Australia, also failed to establish a definite correlation between drought resistance and water requirement. He found that different varieties of wheat showed little

variation in water requirement when the latter was calculated on the basis of total dry weight. Greater differences were noted when water requirement was calculated on weight of grain only. Dillman (14) found small but significant differences in water requirement of different varieties of the same crop, but did not consider them large enough to be used as a basis of selection.

A great deal of literature (15, 19, 26, 37, 44, 50) is available concerning the morphological and anatomical peculiarities which distinguish typical xerophytes. This literature is too extensive to be reviewed in this paper. It should be explained, however, at this point that earlier investigators believed that a drought-resistant structure functioned only in restricting transpiration. Maximov (26) holds the view that the study of xeromorphic structures is only useful when an attempt is made to correlate them with some corresponding physiologic change which may be important in drought resistance.

A number of investigators have studied the feasibility of selecting drought-resistant varieties on the basis of xeromorphic structures that have been induced under drought conditions. This method of approach in regard to selection has been advocated by Maximov (26). Probably the first work to be reported along this line was that of Kolkunov (22). He studied stomatal size in a number of wheat varieties possessing varying degrees of drought resistance, and found the more resistant varieties to be characterized by small stomata. He also found that in humid years the yield of sugar beets and corn was directly proportional, and in dry years inversely proportional to the size of the plant cell. Kolkunov was, however, working under the assumption that transpiration as regulated by the stomata was a good criterion of drought resistance. Yakushkina and Vavilov (26) working with 17 pure lines of different varieties of oats, found no correlation between cell size and yield of crop under humid conditions. Later Kolkunov (26) selected four pure lines of Beloturka wheat differing in cell size, and grew them under different soil moisture contents. He found that under conditions of high soil moisture, the larger celled varieties gave the highest grain yields, while the reverse was true in the case of low soil moisture conditions. Vasiliev (46) obtained no relation between length of stomata and size of cells in wheat. Pavlov (33) reported that, in general, the more drought-resistant and early varieties of winter wheat were characterized by small stomata, but this condition was not apparent in spring wheat and oats. Alexander (5) found a correlation in oats between root and shoot length in the seedling stage and drought resistance. The more drought-resistant varieties produced a relatively smaller proportion of root and shoot at all soil moisture contents, in comparison with the less resistant varieties. Recently Bolsunov (9), Snoep (42) and Pavlov (33) have reported that an index of drought resistance may be obtained by studying the osmotic pressure of germinating seeds, as determined by salt or sugar solutions. Seeds selected for high osmotic pressure gave higher yields than unselected material. Bolsunov (9) found this increase in yield to hold true, under both humid and arid conditions.

Working with the viewpoint of Maximov (26), that the only true criterion of drought resistance is the ability of the plant to endure drought without injury, several Russian investigators have tested the ability of a number of crop plants to resist drought. Tumanov (44) used percentage plant survival as an index of drought resistance, when testing the reaction of eight varieties of wheat to endure a prolonged period of soil drought. He found that varieties exhibiting drought resistant properties in field tests had a survival of over 80%, whereas varieties not exhibiting these properties had a survival of 50% or less. Later Tumanov (45) studied the relative drought resistance of a number of crop plants, including oats, corn, sorghum and alfalfa. The percentage of dead leaves after eight days without available water was used as a measure of drought resistance. He concluded that percentage of dead leaves was not always a good measure of drought resistance, since the degree of resistance of separate organs varied within different plants. Kondo (23), in a study of the resistance of sunflowers and soybeans to soil drought, used both percentage of dead leaves and reduction in grain yield as criteria of drought resistance. Krasnosselsky-Maximov (24) also used these two measures of susceptibility when estimating the injury occurring to cereals as a result of exposure to artificial dry winds.

Physico-chemical studies regarding the nature of drought resistance is the most recent phase of the problem to receive attention. Newton and Martin (32) have studied this phase of the problem for a number of crop plants. Osmotic pressure of tissue fluids was found to vary with the physiological scarcity of water, but was not considered a good index of drought resistance. Bound water content of the cell sap gave more satisfactory results, for it was possible to class a number of cultivated wheats and grasses in order of their drought resistance. These authors suggest that it is the high colloidal content of the cells, which reduces the abstraction of water from them, that accounts for the lack of injury shown in drought-resistant plants. Vasiliev (47), who experimented with wheat plants, believes that sugars play an important role in the protection of plants from desiccation. As drought conditions increase in intensity, increasing amounts of starch are converted into sugar. When water conditions improve, the process is reversed, the soluble carbohydrates decreasing and the insoluble carbohydrates increasing in quantity.

Materials and Methods

Serving as standards of comparison, two Russian wheat varieties of reputed drought-resistant qualities, Milturum 0.321 and Caesium 0.111, introduced from Russia in 1928, have been included in all the studies reported in this paper. These varieties have also exhibited drought resistance in trials conducted at the University of Alberta.

The soil used throughout these investigations, unless otherwise stated, was a brown transitional type between the black and the gray soils obtained from Stony Plain, Alberta. This soil has the same physical properties as the brown soil that is typical of the drought areas of southern Alberta.

In all pot-work reported, six-inch porous clay pots were used. Five kernels per pot were sown. The individual plant was used as a basis of estimating drought injury. In interpreting the results, however, the average injury occurring to the plants in each treatment was used.

In the root studies reported, the root material was extricated from the soil mass by washing with a spray of water from the nozzle of a garden hose. After the root system had been carefully extricated it was floated on the surface of water to facilitate examination and collection on blotting paper and preservation, as shown in Fig. 5.

The studies on the reaction of various wheat varieties to atmospheric drought were made in a wind or "chinook" machine. This apparatus has been described in detail by Aamodt (1, 2). Atmospheric drought, similar to the conditions in the dry area of Alberta when growing grain is severely injured, was produced artificially in a glass tunnel. Wind was blown through the tunnel over the plants at a rate of approximately six miles per hour, at a temperature of 110° F., and a relative humidity of about 15%.

Experimental Results

1. Susceptibility of Wheat Varieties to Drought at Different Stages of Development

The purpose of this study was to determine the relative effects of soil and atmospheric drought on wheat varieties when exposed in the four growth stages, stooling, shooting, soft dough and hard dough. The effects of two soil types were also compared—a black chernozem soil, typical of the Edmonton district, and the transitional type. Two drought-resistant varieties, Milturum and Caesium, and two non-drought-resistant varieties, Marquis and Reward, were used in this study. The plants were grown in six-inch pots with optimum soil moisture conditions. Triplicate series of pots, with one series of three pots serving as a check, were used.

Plants subjected to the soil drought were deprived of water as they reached the stage of development selected for the test, and water was withheld until the plants showed evidence of extreme wilt. They were kept in this wilted condition for three days. Water was then applied to the soil to bring it up to the optimum moisture condition.

The plants subjected to atmospheric drought were given an eight hour exposure in the chinook machine at a temperature of 110° F. and with humidity ranging from 14–17%. The plants received no water during the eight hour exposure.

The percentage leaf loss of the four varieties tested for the stooling and shooting stages is given in Table I. It is necessary to explain at this point that, owing to natural maturation processes, the plants of all varieties when exposed to drought in the soft- and hard-dough stages lost all of their leaves. The phenomenon was similar to that occurring in naturally ripening grain, in that the straw tended to bleach and leaves became brown and desiccated. The leaves showed little evidence of true windburn injury. For this reason the data in Table I pertain only to the stooling and shooting stages.

The data on degree of shrivelling of the kernels resulting from exposure of plants to drought at the four stages of development are summarized in Table II. The degree of kernel shrivelling was estimated by placing the grain from each sample in one of 10 classes. Extremely shrivelled kernels were placed in Class 1, and plump kernels in Class 10. In the studies on breeding for drought resistance in spring wheat, a more accurate determination of kernel plumpness was desired than that usually obtained by taking weight per 1000 kernels. A method was developed for obtaining weight per measured bushel of grain from individual wheat plants (3). The degree of association between weight per measured bushel, obtained by the above method, and an estimated degree of kernel plumpness was very high.

The data given in Table I are based on the average injury shown by the five plants in each pot. The yield of grain from individual plants was too

TABLE I

THE COMPARATIVE RESISTANCE TO ATMOSPHERIC AND SOIL DROUGHT OF FOUR VARIETIES OF WHEAT GROWN IN BOTH BROWN AND BLACK SOILS WHEN EXPOSED AT THE STOOLING AND SHOOTING STAGES

Variety	Percentage leaf loss by							
	Atmospheric drought				Soil drought			
	Stooling stage		Shooting stage		Stooling stage		Shooting stage	
	Brown soil	Black soil	Brown soil	Black soil	Brown soil	Black soil	Brown soil	Black soil
Marquis	95	95	60	*100	5	5	70	40
	85	50	*100	*100	trace	5	50	40
	*100	*100	85	*100	trace	5	50	40
Reward	90	25	*100	95	trace	20–25	90	85
	50	25	*100	*100	10	20	85	40
	90	25	95	95	10	20–25	85	100
Milturum	50	20	*100	100	trace	10–15	50	20
	40	20	100	95	trace	10–15	40	20
	30	20	*100	95	5	10–15	40	20
Caesium	10	10	65	100	trace	20	60	40
	15	10	*100	100	15	10–15	60	35
	10	10	*100	100	15	10–15	75	20

Plants completely killed.

small to permit a reliable estimate to be made of the degree of kernel shrivelling. An estimate of the degree of kernel shrivelling was made on the bulked grain from all of the plants in each treatment. The data are given in Table II.

From a consideration of the data in Table I, it is evident that under the conditions of the experiment much greater injury to the plants resulted from atmospheric drought than from soil drought in the stooling and shooting stages. The effect of atmospheric and soil drought on kernel shrivelling was approximately the same in the stooling stage of development (Table II).

Atmospheric drought was far more severe than soil drought in the shooting stage, as is shown by the percentage of leaf loss (Table I) and the lack of kernel development (Table II).

TABLE II

AVERAGE DEGREE OF SHRIVELLING OF KERNELS OF FOUR VARIETIES OF WHEAT GROWN IN BROWN AND BLACK SOILS WHEN EXPOSED TO ATMOSPHERIC AND SOIL DROUGHT AT FOUR STAGES OF DEVELOPMENT

Variety	Average degree of kernel shrivelling*																	
	Check		Atmospheric drought								Soil drought							
			Stooling		Shooting		Soft dough		Hard dough		Stooling		Shooting**		Soft dough		Hard dough	
	Br.†	Bl.†	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.
Marquis	9	10	9	9	7	0	2	3	6	9	10	10	10	8	2	2	10	5
Reward	9	9	9	9	0	0	8	9	8	9	9	8	9	6	2	6	9	6
Milturum	9	8	9	9	0	0	3	5	10	9	10	7	7	5	4	6	10	7
Caesium	10	9	9	9	0	0	3	2	9	9	9	8	9	7	5	4	9	8

Scale of 1 = shrivelled to 10 = plump; 0 = no seed formed.
†*Br. = brown and Bl. = black soil.*
***Kernels produced largely from second growth.*

Exposure of plants to drought in the stooling stage resulted in injury of a temporary nature, as plants injured at this time made relatively rapid recovery and produced spikes bearing normal plump kernels. The rate of recovery was found to be roughly inversely proportional to the amount of leaf injury. Marquis, Reward and Milturum made a slower rate of recovery after exposure to atmospheric drought than after exposure to soil drought. Generally it was observed that, if foliage injury was not too severe, plants exposed in the stooling stage recovered sufficiently rapidly to mature only a few days later than the unexposed checks. Hence it is felt that plants injured by drought in the stooling stage under field conditions, and in areas of a moderately long growing season, will have considerable economic value, if favorable conditions prevail in the later stages of growth.

Much greater leaf loss resulted when plants were exposed to drought in the shooting stage. This was accompanied by greater culm injuries and a much slower rate of recovery. Culm injury was particularly severe in plants subjected in this stage to atmospheric drought, and resulted in the death of a considerable number of the plants and in almost complete sterility of those which survived. Plants exposed to soil drought in the shooting stage, while exhibiting comparatively high leaf loss, recovered sufficiently to produce culms bearing fairly plump kernels. However, it is necessary to point out in this connection that the greater part of the spikes reaching maturity were produced from second growth, and were much later in maturing than those of the check plants, or those of plants subjected to atmospheric drought during the stooling stage. It is obvious that plants forced to produce new culms after having once reached the shooting stage would have little economic value.

Great reduction in kernel yield due to shrivelling occurred when plants were subjected to drought (either soil or atmospheric) in the soft-dough stage; whereas, after the kernels had reached the hard-dough stage, they were only slightly affected by drought.

The effect of soil type on the degree of plant injury arising from drought was different in the different stages of development. Plants grown in brown soil showed greater consistent leaf losses than those grown in black soil, when exposed to atmospheric drought during the stooling stage and to soil drought in the shooting stage (Table I). The reverse was true when plants were subjected to soil drought during the stooling stage.

2. Hardening of Wheat Plants as a Means of Increasing Their Resistance to Atmospheric Drought

Many plants have the inherent ability to adjust themselves physiologically to adverse conditions when exposed gradually to unfavorable factors. This phenomenon is well illustrated by the work that has been done on the testing of varieties of winter wheat for resistance to low temperatures. The inherent potentialities of varieties to withstand injury from exposure to low temperatures are more clearly differentiated after a "hardening-off" process than when not so treated. General observations and reports in the literature indicated that limited exposures of plants to drought developed a greater degree of tolerance to more severe exposures later on. Studies of the influence of the hardening process on varietal reaction to atmospheric drought were carried out, using the two drought-resistant varieties, Milturum and Caesium, and the two susceptible ones, Marquis and Reward. In the first experiment the plants were grown in wooden boxes holding approximately one cubic foot of soil. The boxes were placed outdoors, where the plants were exposed to the natural atmospheric conditions. Two rows of plants were grown in each box, the rate of seeding being equivalent to one and one-half bushels per acre. Each treatment was carried out in duplicate.

The hardening process consisted in allowing the soil in the boxes to dry out until the plants showed signs of severe wilting, *i.e.*, cell injury of the leaves just beginning to be apparent. The moisture in the soil at this time was considerably below 20% of the water holding capacity. Moisture was then added to the soil and the plants were allowed three days to regain their normal condition. They were then given an eight hour exposure in the "chinook" machine at 110° F., 16% relative humidity and a wind velocity of six miles per hour. At the time of the exposure to windburn, Milturum and Caesium were in the late stooling stage, while Marquis and Reward had just commenced to shoot. The check plants, which had received optimum moisture at all times and consequently were not hardened, were given a similar exposure.

The results of this first experiment are given in Table III. They show clearly the greater resistance to windburn of hardened plants of all four varieties as compared with those not receiving the preliminary treatment.

One important feature of the data in Table III is the high percentage of culm injury shown by the non-hardened plants of Marquis, Reward and Caesium. Culm injury is obviously a very serious matter, as plants injured in this way make slow, if any, recovery. In this experiment no recovery occurred among the non-hardened plants of Reward. Milturum, on the other hand, made comparatively rapid recovery; whereas Marquis and Caesium

TABLE III

LEAF AND CULM INJURY TO FOUR VARIETIES OF WHEAT WHEN EXPOSED TO ATMOSPHERIC DROUGHT WITH AND WITHOUT PREVIOUS HARDENING

Variety	Stage of growth	Degree of injury			
		Not hardened		Hardened	
		% leaf loss	% culm loss	% leaf loss	% culm loss
Marquis	Early shooting	95 85	100 40	40 25	0 0
Reward	Shooting	100 100	100 100	10 + 50 chlorosis	0
Milturum	Late stooling	20 + 50 chlorosis	0	trace	0
		15 + 15 chlorosis	0	trace	0
Caesium	Late stooling to early shooting	75 85	25 75	20 60 chlorosis only	0 0

were intermediate. The injured plants of the hardened lots made rapid recovery in all cases, undoubtedly because of lack of serious culm injury. A number of leaves were found to exhibit chlorosis. When this condition was severe, death of the leaf followed, and when mild, the leaves recovered and appeared normal. Thus a considerable number of the leaves on non-hardened Milturum plants, as well as on the hardened Reward plants, eventually died. Fig. 1 shows a comparison of the hardened and non-hardened plants of Reward and Milturum approximately one month after exposure.

As a result of culm injury, a difficulty arises in assigning injury percentages to the leaves. It is evident that the death of a culm results in death of all the leaves associated with it. However, notations made on leaf injury a few days after exposure to the hot wind often show only a portion of the leaf to be actually injured. Since a period of two to three weeks is necessary before death of the culm is complete, the question arises whether percentage leaf loss should be limited to that arising directly from leaf burn, or whether that associated with death of the culms should also be included. In the present study leaf injury was estimated a few days following exposure; or, in other words, it was calculated independently of loss due to death of the culms.

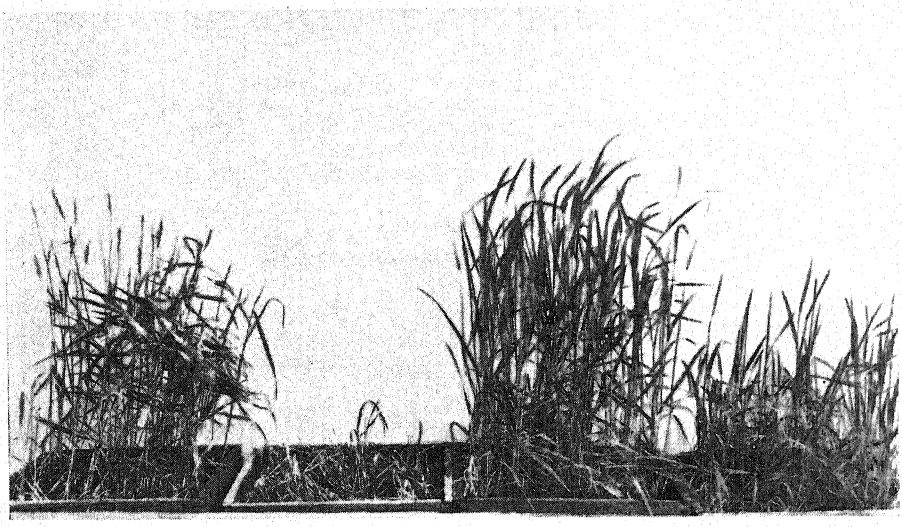

FIG. 1. *Effects of hardening, by soil drought, on the ability of two wheat varieties to withstand atmospheric drought. Left to right: Reward—hardened; Reward—non-hardened; Milturum—hardened; Milturum—non-hardened. (Photograph taken 1 month after exposure).*

The data in Table IV summarize the results of a second experiment, in which Marquis and Milturum were tested in a manner similar to that described above. The plants of these varieties were in the late stooling to early shooting

TABLE IV

PERCENTAGE OF LEAF INJURY TO TWO VARIETIES OF WHEAT WHEN EXPOSED TO ATMOSPHERIC DROUGHT, WITH AND WITHOUT PREVIOUS HARDENING

Variety	Stage of growth	Leaf loss due to soil drought, %	Leaf loss due to atmospheric drought	
			Non-hardened, %	Hardened, %
Marquis	Early shooting	15	50	20
		15	25	30
Milturum	Late stooling	5	85	0
		15	60	5

stages. It will be noted that a certain amount of injury resulted directly from the hardening process. The difference in leaf loss between the hardened and unhardened plants of Marquis is not so striking as in the former experiment. However, that manifested by Milturum is even greater. Little evidence of culm injury was observed in this experiment. The comparatively heavy leaf loss shown by the unhardened plants of Milturum can be attributed only to experimental variation.

The boxes used in the above two experiments were difficult to handle in the chinook machine. Also the number was necessarily limited because of

the size. Consequently it was thought advisable to study the effects of hardening plants grown in ordinary six-inch pots. Marquis and Milturum were selected for the experiment. Ten pots of five plants each were used in each treatment. The plan of the experiment was as follows:

1. Check plants received an eight hour exposure in the chinook machine without previous hardening.

2. Plants hardened by two consecutive exposures of three and a half hours each in the chinook machine, followed by a final exposure of eight hours.

3. Plants hardened by exposure to soil drought (water holding capacity approximately 15%), followed by an eight hour exposure in the chinook machine.

After each hardening treatment, the plants were watered and allowed sufficient time to recover to approximate normality. Owing to considerable variation among plants within each treatment, the average percentage leaf loss occurring in the plants contained in each series of 10 pots was used to interpret the results.

The results of this experiment, as shown by the data in Table V, again illustrate the relative resistance to windburn of hardened plants as compared with those not receiving this treatment. The least leaf loss occurred in the

TABLE V

PERCENTAGE LEAF LOSS OF TWO VARIETIES OF WHEAT GROWN IN POTS AND EXPOSED TO ATMOSPHERIC DROUGHT FOR EIGHT HOURS WHEN NON-HARDENED, WHEN HARDENED BY LIMITED EXPOSURES TO ATMOSPHERIC DROUGHT, AND WHEN HARDENED BY SOIL DROUGHT

Variety	Check		Hardened by atmospheric drought		Hardened by soil drought	
	Leaf loss, %	Leaves chlorotic, %	Leaf loss, %	Leaves chlorotic, %	Leaf loss, %	Leaves chlorotic, %
Marquis	80	15	30	trace	9	0
Milturum	35	22	12	trace	11	0

plants hardened by exposure to soil drought. The plants hardened by short exposures to atmospheric drought showed little leaf wilt after the first hardening exposure, but exhibited considerable chlorosis. The combined percentages of leaf wilt and chlorosis very closely approximated the total leaf injury noted after the final eight hour exposure. This indicates that very little additional injury occurred to the leaves following this initial treatment. A greater leaf loss at the termination of the experiment was due to a more thorough wilting of the already chlorotic leaves. There was no additional injury to the leaves after the second hardening exposure. This may be explained by assuming that the duration of the exposure at that time was not sufficiently long to bring about further leaf injury. Fig. 2 shows the degree of injury occurring

in Marquis plants hardened by short exposures in the chinook machine, when given a severe exposure to atmospheric drought, compared with plants not hardened.

FIG. 2. *Effects of hardening, by short exposures to atmospheric drought, on the ability of Marquis plants to withstand a long exposure to atmospheric drought. Four pots on left, hardened. Four pots on right, non-hardened.*

FIG. 3. *A comparison of the ability of two varieties of wheat to recover from exposures to soil drought. Two pots on left, Reward; two pots on right, Caesium. (Photograph taken two weeks after rewatering).*

Reward was included in one of the experiments in which the plants were to be hardened by soil drought previous to exposures to atmospheric drought. It appeared to be especially susceptible to soil drought as compared with Caesium as is shown by the following observation. Plants of Reward and Caesium were grown in pots in which the soil moisture was allowed to reach a point which caused the plants to wilt. When the moisture was again added to the soil, Caesium made rapid recovery and soon showed normal leaf development. Reward failed to recover and eventually died. The appearance of the two varieties two weeks after re-watering is shown in Fig. 3.

Methods 3. VARIETAL REACTION TO ATMOSPHERIC DROUGHT

The varieties selected for a preliminary study of varietal reaction to artificially produced drought are as follows: Baart and Federation, two soft wheats, reported as exhibiting qualities of drought resistance in the United States and Australia; Pentad and Kubanka, two durum wheats, included in the study owing to certain conflicting reports concerning the drought-resisting properties of durum wheats; Milturum, a drought-resistant Russian wheat; Canus, a promising new variety developed at the University of Alberta; Red Bobs No. 222, a relatively early, high yielding, common wheat.

In experiments with these seven varieties, each variety was replicated three times in six-inch pots. All exposures in the chinook machine were made after the plants had reached either the late stooling or early shooting stages. The temperature prevailing during the exposures was 110° F., the relative humidity varied from 13–18%, and the wind velocity from six to seven miles per hour.

In the first experiment the plants were exposed for eight hours in the chinook machine, without previous hardening. After this treatment the plants were allowed five days to recover with an optimum soil moisture content. They were then re-exposed for 15 hours. After each exposure the percentage of leaves killed was estimated and recorded; also the percentage of plants killed and the percentage of culms killed on the plants that survived. The data are given in Table VI. These represent averages based on the total number of plants of each variety used per treatment.

In the second experiment the plants were hardened by permitting them to wilt temporarily as a result of soil drought. After this preliminary hardening process the plants were allowed to recover, and they were then exposed to atmospheric drought in exactly the same manner as the plants in the first experiment. After a period of two weeks the plants were given a third exposure to atmospheric drought for 15 hours. The injury to the plants was estimated as in the first experiment. When the plants began to recover after the third exposure a number of new shoots developed. Notes on new shoot development were also recorded. The data obtained in this experiment are summarized in Table VII.

In the third experiment, the plants were given a single exposure of 15 hours to atmospheric drought, without previous hardening. The data obtained are given in Table VIII.

The modifications in the manner of conducting these experiments were made in an attempt to parallel as closely as possible natural occurrences in the field. Under natural conditions, plants are seldom subjected to sudden periods of hot dry winds, but are usually "hardened off" by gradually increasing temperatures, or by limited soil moisture. Similarly, they are seldom exposed to a single period of hot wind, but usually have to weather several hot periods in a single growing season.

Results

The results of the first experiment, summarized in Table VI, show the varieties tested to fall into three groups as regards susceptibility to atmospheric drought. The first of these, comprising the susceptible varieties, includes the

TABLE VI

COMPARATIVE INJURY TO SEVEN VARIETIES OF WHEAT AFTER TWO EXPOSURES TO ATMOSPHERIC DROUGHT WITHOUT PRELIMINARY HARDENING OF THE PLANTS

Variety	Total number of plants	Total leaf loss in per cent after—		Plants killed, %	Culms killed, %
		First exposure (8 hr.)	Second exposure (15 hr.)		
Federation	15	47	65	27	42
Baart	15	25	80	73	77
Red Bobs No. 222	17	5	62	12	43
Kubanka	15	15	100	100	100
Pentad	15	10	88	47	57
Canus	16	trace	15	0	0
Milturum	16	trace	20	5	0

two durums Kubanka and Pentad, and the soft wheat Baart. Of these, Kubanka shows the greatest injury, with 100% leaf, culm and plant loss. Pentad and Baart exhibit an equal leaf loss of approximately 80%, but differ somewhat in the percentage of dead culms and plants. Baart shows the greater injury in these respects. Federation and Red Bobs No. 222 fall into a second group, and may be termed moderately susceptible. The varieties exhibited approximately 60% loss of leaf and 40% of culm. Federation showed a greater number of dead plants than Red Bobs No. 222. The resistant group, consisting of Milturum and Canus, exhibited less than 25% leaf loss and little injury of the culms.

It will be evident that the ranking of the varieties with regard to percentage leaf loss, following the initial exposure of eight hours, is not in direct agreement with that shown after two successive exposures. Kubanka and Pentad show comparatively less injury and Federation comparatively more after the first

exposure than they do after the two successive exposures. Evidently a single exposure of eight hours is not sufficient to bring out true varietal differences.

The results of the second experiment (Table VII) show up essentially the same varietal differences as the first. The injury exhibited by the plants in this experiment is not as severe as in the former, no doubt owing to the initial hardening treatment given the plants. A certain amount of re-stooling of the plants occurred following the three consecutive exposures. Baart, Red Bobs No. 222 and Milturum showed the greatest tendency in this regard.

TABLE VII

COMPARATIVE INJURY TO SEVEN VARIETIES OF WHEAT AFTER PRELIMINARY HARDENING AND EXPOSURES TO ATMOSPHERIC DROUGHT

Variety	Total number of plants	Total leaf loss in per cent shown after—			Plants killed, %	Culms killed, %	Average number of new shoots per plant
		First exposure (8 hr.)	Second exposure (15 hr.)	Third exposure 15(hr.)			
Federation	14	trace	22	32	8	10	0.8
Baart	15	trace	23	37	0	23	1.5
Red Bobs No. 222	14	trace	20	30	0	0	1.2
Kubanka	15	5	37	60	33	38	0
Pentad	15	5	27	58	33	42	0.3
Canus	15	trace	17	25	0	5	0.3
Milturum	15	trace	7	15	0	0	1.3

With the exception of Red Bobs No. 222, which showed comparatively greater injury, the behavior of the varieties to a single exposure of 15 hours, (third experiment, summarized in Table VIII) was in general accord with those of the two former experiments. No explanation can be given other than that Red Bobs is particularly susceptible to long exposures in the unhardened condition. It will be noted that no culm loss occurred in the case of the varieties Canus and Milturum, while the smallest loss in the other varieties was 22%.

TABLE VIII

COMPARATIVE INJURY TO SEVEN VARIETIES OF WHEAT AFTER A FIFTEEN HOUR EXPOSURE TO ATMOSPHERIC DROUGHT WITHOUT PRELIMINARY HARDENING OF THE PLANTS

Variety	Total number of plants	Loss in per cent		Plants killed, %	Average number of new shoots per plant
		Leaves	Culms		
Federation	15	37	22	0	0.1
Baart	17	45	37	0	0.3
Red Bobs No. 222	11	55	43	18	1.0
Kubanka	15	75	43	40	0
Pentad	15	90	83	80	0
Canus	15	25	0	0	1.0
Milturum	15	20	0	0	1.3

Consistent specific and varietal differences in regard to resistance to wind-burn injury are apparent from the results of these three experiments. The two durum varieties Pentad and Kubanka, and the soft wheat Baart, appeared particularly susceptible, as judged by leaf, culm and plant loss. Federation and Red Bobs No. 222 evinced susceptibility but were not as severely injured as the former. Milturum and Canus, on the other hand, showed considerable resistance (See Fig. 4). These varieties exhibited comparatively small percentages of leaf and culm loss and, for this reason, showed rapid recovery following exposure to drought.

FIG. 4. *Reaction of six wheat varieties following two successive exposures of 8 and 15 hours to atmospheric drought. No previous hardening. Back row, left to right;—Red Bobs No. 222, Canus, Milturum. Front row, left to right;—Kubanka, Baart, Pentad.*

There was fairly good agreement between the different measures of injury used. Thus the varieties Pentad, Kubanka and Baart, which ranked highest in leaf loss, also ranked highest in per cent dead culms and per cent dead plants. On the other hand the low leaf loss exhibited by Canus and Milturum was accompanied by a correspondingly low loss of culms.

General observations which could not be readily expressed in tabular form suggested that there were two aspects of the resistance shown by Milturum. There was an inherent resistance of the cells to desiccation and a remarkable capacity on the part of the plant to produce new leaves and shoots. Canus, while not showing any marked capacity to produce new leaves, showed a high resistance to desiccation which allowed the plants to develop uninterrupted by exposure to the hot winds. The surviving plants of the other varieties tested, particularly those of Pentad and Kubanka, often failed to regain normal vigor. In many cases only one culm reached maturity. Many

of these mature culms showed abnormalities, being so bent at the nodes as to be almost recumbent.

In view of the great susceptibility to windburn shown by the two durum varieties, the findings of certain Russian investigators are interesting (34). Varieties of *T. durum* were found to be characterized by considerable drought resistance during the period of ripening, but did not show this characteristic in the initial growth stages. The present work shows the extreme susceptibility of durums to atmospheric drought in the earlier stages.

The beneficial effects of hardening the plants as a means of rendering them more tolerant to the effect of atmospheric drought is clearly evident from a comparison of the data contained in Table VI (compiled from unhardened plants), and those in Table VII (compiled from hardened plants). Much greater leaf loss occurred after comparable exposures when the plants were unhardened than when they were hardened. The hardened plants also exhibited a considerably lower percentage of dead culms and plants.

4. Studies on Primary Root Development of Wheat Varieties

Varietal Differences

Talanov (43) states that one of the important contributory factors to the drought resistance possessed by Milturum and Caesium lies in the character of their root systems. These varieties have the capacity to develop their root systems rapidly during the early stages of growth before full tillering. The root systems are also greater in their ultimate aspect in that they are profusely branched. Talanov believes that the root systems of these varieties absorb water from a greater volume of soil (up to the limiting minimum connected with the peculiarites of the soil).

In view of Talanov's work, it was decided to commence a study of the early root development of some of the varieties commonly grown in western Canada, and also of certain drought-resistant varieties. In a preliminary experiment conducted during the summer of 1933 root studies were made of Milturum, Caesium, Reward and Marquis. The plants were grown in boxes (1 cu. ft.) placed outdoors. Two rows of 35 seeds (rate equivalent to one and one-half bushels per acre) were sown per box. There were four replicates of each variety, two to be examined for root development at the end of two weeks, and two at the end of one month.

The first examination of the root systems was made 16 days after emergence, when the plants possessed from five to seven leaves. It must be remembered that at this time no secondary roots were evident, and hence all discussion to follow is concerned only with primary roots. Distinct varietal differences in density of root system were apparent. Milturum and Caesium had developed a much more highly branched system than was evident in Marquis and Reward. The root system of Reward seemed especially lacking in this respect. General observations indicated that, while no great varietal differences were apparent in the number of primary roots produced, the drought-resistant varieties had a slightly larger average number. No differences

existed between varieties in length of root systems, all having reached a length of 12 to 14 inches. For purposes of photography the root systems of 25 random plants were floated on to cardboard, and teased out to form a mat three and a half inches in width. Fig. 5 shows the development of the root systems of the four varieties in the test, 16 days after emergence.

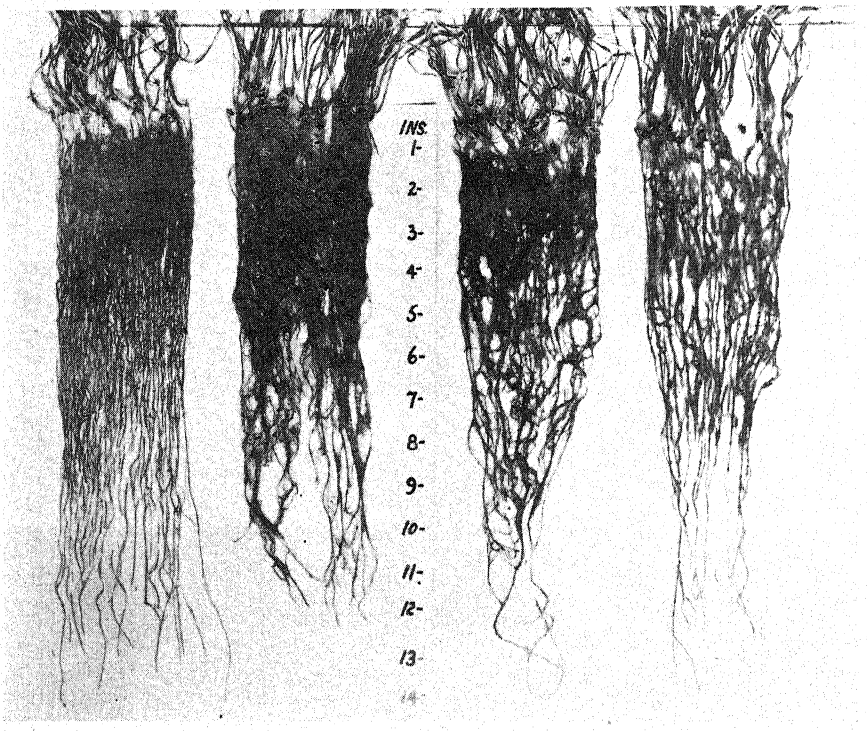

FIG. 5. *Primary root systems of seedlings of drought-resistant and non-drought-resistant varieties of wheat, sixteen days after emergence. Left to right—Milturum, Caesium (drought-resistant), Marquis, Reward (non-drought-resistant).*

Another examination of the roots was made 31 days after emergence. At this time, the secondary roots had made their appearance and were extensive enough to complicate the examination of the primary roots. Furthermore, the primary roots had outgrown their boxes and were badly matted at the bottom, making it difficult to disentangle them. It was quite apparent, however, that the differences noted after two weeks of growth still persisted.

The results of this preliminary experiment were so interesting that arrangements were made to continue these studies in the greenhouse during the winter of 1933–34. For this work, special boxes were constructed, with two removable sides to facilitate the washing out of the roots from the soil. These boxes measured 10 × 10 × 9 in. and contained a little more than one-half a cubic foot of soil. Two rows of 25 seeds were sown per box, and all studies were carried out in duplicate. The varieties used in this study were Reward.

Garnet, Red Bobs No. 222, Marquis, Reliance and Canus; two soft wheats, Baart and Federation; two durums, Pentad and Pelissier; and two with drought-resistant properties, Milturum and Caesium. Wild oats were also included in this study.

Before discussing the results of this experiment, it is necessary to make some comment on the unsuitable growing conditions prevailing during the months of November and December, the period in which the plants were growing. During this interval of time the amount of sunlight was greatly limited by cloudy weather and by low outdoor temperatures that kept the glass covered with frost. This resulted in spindly, weak plants, lacking the healthy green color of normal seedlings. At the end of 23 days following emergence, little stooling of the plants was evident and only three or four leaves had formed. Root studies made at this time soon revealed the fact that root development had also been adversely affected. The development was scarcely more than half of that shown by plants grown outdoors in the summer. The method of study as originally planned consisted in recording the number of primary roots produced by each plant, and in devising a scale for estimating the degree of branching of these roots. It was found impossible, however, to do more than simply estimate the degree of branching, owing to the great variation between plants in the same box. This variation appeared to be due to the caking of the soil, which checked the normal growth of the roots downward, resulting in a well branched, but short, root system.

A summary of the results obtained in this study is to be found in Table IX. The data are expressed as an average for the total number of plants in each treatment. Distinct varietal differences in the number of primary roots developed are apparent. Under the conditions of the experiment Pelissier and Pentad, the two durums, distinctly excelled in this regard, having an average of practically five primary roots per plant. Milturum, Caesium and Baart had an average of over four roots per plant, while Marquis, Red Bobs No. 222 and Garnet, the next three varieties of merit, all had an average of only slightly over 3.6 roots per plant. Reward, Federation and Canus average only slightly over three roots per plant. Of the wheat varieties tested, Reliance shows the smallest number of primary roots, averaging only three roots

TABLE IX

COMPARATIVE PRIMARY ROOT DEVELOPMENT OF SEEDLINGS OF TWELVE VARIETIES OF WHEAT AND WILD OATS TWENTY-THREE DAYS AFTER EMERGENCE

Variety	No. of primary roots per 100 plants	Branching
Pelissier	499	good
Pentad	485	good
Milturum	441	good
Caesium	406	good
Baart	402	poor
Red Bobs No. 222	365	fair
Garnet	364	fair
Marquis	362	fair
Reward	341	poor
Federation	333	poor
Canus	321	fair
Reliance	304	excellent
Wild oats	300	poor

per plant. The root system of this variety, however, excelled all others in the degree of branching. It will be noted that of the other varieties only Milturum, Caesium and the durums showed well branched root systems. Baart, Reward, Federation and wild oats were distinctly lacking in this regard; while Marquis, Red Bobs No. 222, Garnet and Canus can be classed only as fair. Wild oats averaged only three primary roots per plant.

In view of the unfavorable growing conditions prevailing during this experiment, it would seem advisable not to attach too much significance to the above data. However, from results of a later experiment, carried out under more favorable conditions (reported in the next section of this paper), it was found that the branching of the roots was handicapped to a much greater degree than was the actual capacity to produce primary roots. Hence it may be concluded that, from the point of view of primary root development, the varieties are listed in Table IX in approximately the correct order. Further work in regard to varietal differences in the degree of branching of the roots is necessary.

Effect of Phosphorus on Primary Root Development

The general plan of this experiment differed from the one above, in that only the four varieties Reward, Marquis, Milturum and Caesium were used, and that superphosphate was added to the soil of one series of boxes. The rate was approximately 300 lb. per acre. Growing conditions in the greenhouse had improved greatly in the meantime, which was reflected in increased vigor of the seedlings. There was a very close agreement in the number of primary roots in the duplicates. The effect of phosphorus on the development of primary roots is shown by the data in Table X.

It is evident that with the exception of Reward, the addition of phosphorus did not stimulate primary root production, but, on the contrary, seemed to check such a tendency. A comparison of the primary root development of the varieties Marquis, Milturum and Caesium from untreated boxes, as shown in Table X, with that shown by the same varieties in Table IX, revealed little difference in number of primary roots. Evidently the growing conditions in the two experiments had little influence on the formation of the number of primary roots produced by these varieties. A similar comparison in the case of Reward indicates that the growing conditions prevailing in this experiment had some

TABLE X

EFFECT OF SUPERPHOSPHATE ON PRIMARY ROOT DEVELOPMENT OF WHEAT SEEDLINGS

Variety	Treatment	Number of primary roots per 100 plants			Increase or decrease of treatment over check
		1	2	Ave.	
Reward	P	420	448	434	
	No P	406	411	409	+25
Marquis	P	332	328	330	
	No P	340	336	338	− 8
Milturum	P	450	440	445	
	No P	458	466	462	−17
Caesium	P	407	421	414	
	No P	433	445	439	−25

effect on the primary root formation of this variety. Under these conditio there appeared to be a slight beneficial effect from phosphorus on the develo ment of primary roots in Reward.

A rough approximation of degree of branching of the roots washed out fro phosphorus-treated and untreated soil was obtained by floating the roots o 30 random plants from each treatment on the cardboard, where they coul be examined closely. No stimulatory action of the phosphorus on roo branching was observed.

5. Germination of Wheat in Various Concentrations of Sodium Chloride, Potassium Chloride and Sucrose

Recently a number of investigators have reported that it is possible to select drought resistant strains of different crops by the osmotic pressure exhibited by their germinating seed, as differentiated by salt and sugar concentrations. In order to test the feasibility of this method of determining drought resistance, the kernels of a number of wheat varieties, including drought-resistant and non-drought-resistant types, were germinated in varying concentrations of sugar and salt solutions. For this purpose a germinating tray was constructed by stretching a double layer of cheesecloth over a 10 × 6 × 1 in. wooden frame. The tray was divided by means of tin strips into 12 compartments. A different lot of seed was placed in each compartment. All portions of the tray or vessel likely to rust were given a liberal coating of "asphalt black" paint. The whole tray was then placed in a vessel containing the desired solution.

Experiments were conducted using different concentrations of sodium chloride, potassium chloride and sucrose. Distilled water was used as a check. To keep evaporation at a minimum all germination tests were carried out over water in a galvanized iron humidity chamber. As a further precautionary measure to insure constant concentrations, a change of solution was made after 72 hours. In addition to the two drought-resistant varieties Milturum and Caesium, tests were carried out with Reward, Garnet, Red Bobs No. 222 and Marquis. Two lots of 50 seeds each were used in every test.

The data obtained are given in Tables XI, XII and XIII. It will be seen from a study of the germination percentages in the check solutions that the kernels of Milturum, Caesium and Garnet naturally germinate more rapidly than those of the other three varieties. This rapid germination is apparent in all the concentrations of the chemicals used, but does not appear to increase with increased concentration. In other words, when the concentration of a given chemical is increased the germination of all six varieties is retarded somewhat, but the relative rapidity of germination of the varieties remains

the same. Hence, in the light of these experiments, the drought-resistant properties of Caesium and Milturum cannot be said to be necessarily associated with superior abilities of their kernels to germinate in the more concentrated solutions. It is possible, however, that this superior energy of germination is one of many characteristics of drought resistant varieties. If this be the situation, then Garnet, which cannot be classed as truly drought-resistant, must lack certain other essential properties necessary for drought resistance. It is interesting in this connection to note that Bolsunov (9) found that kernels from wheat strains selected for their ability to germinate in salt concentrations of 10 to 15 atmospheres showed an increased energy of germination, as compared with those from unselected strains.

TABLE XI

A COMPARISON OF GERMINATION PERCENTAGES OF SIX VARIETIES OF WHEAT WHEN TESTED IN VARIOUS CONCENTRATIONS OF SUCROSE ($C_{12}H_{22}O_{11}$)

Concentration of solution	Variety	%Germination at the end of									
		12 hr.	24 hr.	48 hr.	60 hr.	72 hr.	84 hr.	96 hr.	108 hr.	120 hr.	144 hr.
Check—distilled water	Reward	0	0	3	33	58	92	98	—	—	—
.1 *N*		0	0	1	6	18	69	91	98	—	—
.2 *N*		0	0	0	8	20	62	88	91	—	—
.25 *N*		0	0	0	2	15	63	80	86	91	—
.3 *N*		0	0	0	4	15	29	49	85	95	—
Check—distilled water	Marquis	0	0	1	35	66	87	96	—	—	—
.1 *N*		0	0	0	21	40	90	96	98	—	—
.2 *N*		0	0	0	7	36	68	91	94	—	—
.25 *N*		0	0	0	2	19	44	83	90	92	—
.3 *N*		0	0	0	3	14	41	70	85	88	—
Check—distilled water	Red Bobs 222	0	0	1	24	58	96	97	—	—	—
.1 *N*		0	0	1	15	33	81	98	100	—	—
.2 *N*		0	0	0	16	29	71	94	96	—	—
.25 *N*		0	0	0	4	18	39	69	95	97	—
.3 *N*		0	0	0	4	8	23	31	82	98	—
Check—distilled water	Garnet	0	2	6	67	80	98	—	—	—	—
.1 *N*		0	0	1	26	53	95	99	—	—	—
.2 *N*		0	0	0	23	66	93	96	—	—	—
.25 *N*		0	0	0	16	35	72	87	94	95	—
.3 *N*		0	0	0	13	19	56	67	94	97	—
Check—distilled water	Milturum	0	2	7	47	78	96	98	—	—	—
.1 *N*		0	1	3	31	60	94	96	97	—	—
.2 *N*		0	0	0	31	52	89	98	—	—	—
.25 *N*		0	0	0	24	34	71	88	93	96	—
.3 *N*		0	0	0	14	26	52	78	89	94	—
Check—distilled water	Caesium	0	3	7	48	75	100	—	—	—	—
.1 *N*		0	2	7	20	58	97	99	—	—	—
.2 *N*		0	0	0	24	49	86	95	99	—	—
.25 *N*		0	0	0	16	36	76	92	96	98	—
.3 *N*		0	0	0	6	21	39	52	77	94	—

It must be remembered that only "one salt" solutions were used in t present work. For this reason seedlings were unable to develop after t necessary growth elements contained in the kernels were used up. It wo seem that a much more promising method of attacking this problem wo be to make up balanced solutions of varying osmotic pressures. In this w not only could the germination rates be studied, but the seedlings could actually grown in these solutions. This would afford the investigator much greater opportunity of studying the value of osmotic pressures as aid in the selection of drought resistant strains.

A number of investigators (9, 42) have reported good results in the selecti of drought resistant strains of various crops, through the use of salt solutio It is possible that these investigators were selecting from a heterogeneo population of a given variety, and were not concerned with varietal differenc as a whole.

TABLE XII

A COMPARISON OF THE GERMINATION PERCENTAGES OF SIX VARIETIES OF WHEAT WHEN TEST IN VARIOUS CONCENTRATIONS OF POTASSIUM CHLORIDE (KCl)

Concentration of solution	Variety	% germination at the end of							
		12 hr.	24 hr.	36 hr.	48 hr.	60 hr.	72 hr.	84 hr.	96 hr
Check—distilled water	Reward	0	0	69	82	96	—	—	—
.2 *N*		0	0	4	35	64	69	70	—
.4 *N*		0	0	0	0	13	19	33	..
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Marquis	0	1	82	84	94	—	—	—
.2 *N*		0	0	1	10	47	61	77	—
.4 *N*		0	0	0	0	12	22	27	—
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Red Bobs 222	0	0	91	97	—	—	—	—
.2 *N*		0	0	4	22	59	79	94	—
.4 *N*		0	0	0	0	17	21	45	—
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Garnet	0	7	73	93	95	—	—	—
.2 *N*		0	3	23	71	84	84	89	—
.4 *N*		0	0	0	1	21	30	38	—
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Milturum	0	10	79	95	—	—	—	—
.2 *N*		0	4	12	32	68	75	80	—
.4 *N*		0	0	1	4	18	29	38	—
.6 *N*		0	0	0	0	0	0	2	—
Check—distilled water	Caesium	0	12	89	96	—	—	—	—
.2 *N*		0	7	25	60	81	81	84	—
.4 *N*		0	0	1	6	28	38	50	—
.6 *N*		0	0	0	0	0	0	2	—

TABLE XIII

A COMPARISION OF GERMINATION PERCENTAGES OF SIX VARIETIES OF WHEAT WHEN TESTED IN VARIOUS CONCENTRATIONS OF COMMON SALT (NaCl)

Concentration of solution	Variety	% germination at the end of								
		12 hr.	24 hr.	36 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.
Check—distilled water	Reward	0	1	32	94	95	—	—	—	—
.1 *N*		0	2	54	100	—	—	—	—	—
.2 *N*		0	0	10	24	75	89	—	—	—
.3 *N*		0	0	0	6	47	83	93	97	—
.4 *N*		0	0	0	1	4	23	66	81	—
Check—distilled water	Marquis	0	2	28	96	99	—	—	—	—
.1 *N*		0	0	70	97	—	—	—	—	—
.2 *N*		0	0	18	36	86	93	—	—	—
.3 *N*		0	0	0	4	61	86	93	—	—
.4 *N*		0	0	0	1	24	64	76	79	—
Check—distilled water	Red Bobs 222	0	5	31	96	99	—	—	—	—
.1 *N*		0	2	65	95	—	—	—	—	—
.2 *N*		0	1	20	42	95	99	—	—	—
.3 *N*		0	0	0	6	55	91	96	—	—
.4 *N*		0	0	0	5	35	66	89	—	—
Check—distilled water	Garnet	0	9	41	89	96	—	—	—	—
.1 *N*		0	8	96	—	—	—	—	—	—
.2 *N*		0	0	42	64	94	—	—	—	—
.3 *N*		0	0	0	18	86	95	—	—	—
.4 *N*		0	0	0	8	67	88	92	—	—
Check—distilled water	Milturum	0	14	75	96	—	—	—	—	—
.1 *N*		0	0	92	99	—	—	—	—	—
.2 *N*		0	4	38	58	90	98	—	—	—
.3 *N*		0	0	0	15	73	91	93	—	—
.4 *N*		0	0	0	13	69	82	84	—	—
Check—distilled water	Caesium	0	14	73	98	—	—	—	—	—
.1 *N*		0	4	83	97	—	—	—	—	—
.2 *N*		0	4	37	50	88	97	—	—	—
.3 *N*		0	0	0	17	67	97	—	—	—
.4 *N*		0	0	0	8	58	78	85	—	—

Conclusions

The work reported in this paper summarizes a number of attempts to obtain information regarding the nature of drought resistance, particularly as it exists in wheat. The method of attack consisted, in general, in comparing certain physiological and morphological features of the drought-resistant Russian varieties Milturum and Caesium with those of commonly grown susceptible types. These studies have indicated that at least three factors contribute to the drought resistance of these Russian varieties.

In the first place, these varieties have, in contrast to Reward, Marquis, etc., the ability to evade early periods of drought. This characteristic is particularly evident in the case of Milturum. It has been shown in this paper that these Russian varieties possess greater resistance to atmospheric drought

than the commonly grown types, when tested in the stooling stage. This superior resistance is not evident at the critical periods of shooting and heading. The significance of these facts becomes more apparent when it is pointed out that Milturum has approximately 8–10 days and Caesium 4–5 days longer stooling period than Marquis. While in this resistant stage, these varieties are able to survive periods of drought that ordinarily cause great damage to other sorts not possessing this delayed habit of growth. Talanov (43) has reported that the drought resistance shown by Milturum, when growing in certain drought areas of Russia, was due in part to a delayed stooling period.

Milturum and Caesium were also found to possess the capacity of developing their root systems rapidly in the early stages of development. This characteristic is of obvious importance when limited moisture is available at the time of seeding, since it allows the plant to establish itself more quickly in the soil. Furthermore, an early and well developed root system will enable a plant to weather early periods of drought more successfully. The importance of this in connection with soil drought is illustrated in Fig. 3. Reward, which is characterized by a scanty development of root system, was unable to survive a prolonged period of soil drought, while Caesium made complete recovery. Talanov (43) has reported that the root systems of Milturum and Caesium in their ultimate aspect are also characterized by profuse branching and great thickness.

Finally, the Russian varieties were found to possess a superior capacity to endure drought without permanent injury. Tumanov (44) has pointed out that Caesium possesses considerable resistance to soil drought. This investigator found that the wheat varieties that he tested fell into two distinct groups with regard to survival, following a prolonged exposure to soil drought, *viz.*, those with a survival of more than 80% and those with a survival of less than 50%. Caesium fell into the former group.

The results of the present study show Milturum and Caesium to be particularly resistant to atmospheric drought when tested in the earlier stages of development. They not only showed, as a rule, less foliage injury than the susceptible types, but made more rapid recovery. Milturum was superior to Caesium in both these respects.

Hardening of the plants prior to exposure to atmospheric drought, by subjection to soil drought or to limited periods of atmospheric drought, caused greater tolerance in both resistant and susceptible types. Hardening was reflected in a reduced leaf and culm injury, and in more rapid recovery as determined by general appearance of vigor. It was a noticeable feature of the work on hardening that unhardened plants, which did not appear to be unduly injured after exposure, were found to be much more stunted at maturity than corresponding hardened plants. This is well illustrated in Fig. 1, where the striking difference in height of hardened and unhardened plants of the Milturum variety is clearly indicated.

Varietal differences in susceptibility tended to be minimized when the plants were hardened before exposure to drought (See Tables VI–VIII).

It is thought that greater differences would have occurred had the exposures been longer so as to overcome somewhat the tolerating effect of hardening.

Percentage leaf loss was found to be a fairly reliable criterion of susceptibility when the plants were in either the stooling or early shooting stage. While close agreement existed in most cases between per cent leaf loss and per cent of culms and plants killed, one or two exceptions which are worthy of note occurred. With reference to Table VII, it will be noted that the leaf loss of the three varieties, Federation, Baart and Red Bobs No. 222, varied from only 30–37%, while the culm loss for some varieties varied from 0–23%. These data indicate that the most reliable index of susceptibility to atmospheric drought is obtained when per cent leaf loss is estimated in conjunction with per cent dead culms and per cent dead plants.

Leaf and culm loss were found to be of little value as a measure of susceptibility when the plants were tested in the advanced stages of soft and hard dough. At this time the leaves were very prone to desiccation, the phenomenon being not unlike that occurring in naturally ripening grain. The maturation processes in the culms were also hastened, and as they had lost their succulency at these stages it was difficult to distinguish between injured and non-injured culms. The degree of shrivelling of the kernels appeared to be the only reliable criterion of injury at these later stages.

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INVESTIGATIONS RELATIVE TO THE BREEDING OF COUMARIN-FREE SWEET CLOVER, *MELILOTUS*[1]

BY T. M. STEVENSON[2] AND J. S. CLAYTON[3]

Abstract

A series of studies was made upon the coumarin content of various species, varieties and individual plants of sweet clover *(Melilotus)*, determinations being made from different parts of the plant, at different stages of growth, and upon herbage dried by different methods. The conclusions drawn from the results of these studies may be summarized as follows:— (1) Coumarin content of the leaf and stem of sweet clover changes rapidly throughout the various stages of growth. (2) Wide variations in coumarin content exist between different species, between different varieties within a species and often between different individuals within a variety. (3) There is a marked relation between color of leaf and coumarin content. Plants possessing dark-colored foliage have invariably tested higher in coumarin content than those with foliage of lighter color. (4) The Alpha variety possesses a lower coumarin content than any other variety of *M. albus* tested. (5) The species, *Melilotus dentatus*, used in these tests, contained less than 0.01% of coumarin in the foliage and less than 0.05% in the mature seeds. For all practical purposes it may be regarded as coumarin-free. (6) Air drying or oven drying of sweet clover results in a heavy loss of coumarin from the leaves and marked changes in the coumarin content of the stems. (7) There is a definite correlation between the coumarin content of the leaf and of the mature seed in the materials used. (8) The coumarin content of the mature seed provides a reliable estimate of the coumarin content of the leaf of the plant. (9) Breeding results indicate the possibility of producing low coumarin varieties through inbreeding and selection.

Introduction

The investigations discussed in this paper were begun in 1933 and have been continued to the present time. The original purpose of the work was to ascertain facts which might be of assistance in selecting material for use in breeding coumarin-free sweet clover *(Melilotus)* of agricultural value. While, so far as the writers have been able to learn, no exact data are available with respect to the relative values of bitter and non-bitter sweet clover for hay and pasture purposes, there can be no doubt that the elimination of the bitter principle improves palatability. In addition, the possibility of coumarin and related compounds being in some manner associated with the toxic properties of sweet clover has not, as yet, been entirely eliminated. The production of coumarin-free varieties will make it possible to deal with this question more effectively.

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The fact that certain species of *Melilotus* are relatively free from coumarin has been recognized for some time. Kuznetsov (7) describes *M. dentatus* as being almost entirely free from the odor of coumarin, or as containing very small amounts of this substance. Unfortunately, however, this species possesses certain other characters which make it relatively undesirable as a cultivated plant. It therefore seems desirable to seek non-bitter types in species of greater agricultural importance.

In this study an effort has been made to determine: (a) the relative proportions of coumarin present in the stems and leaves of sweet clover at various stages from early growth to maturity, (b) what correlation, if any, exists between the amount of coumarin present in the leaves or stems and the mature seeds of the same plant, (c) the variation in coumarin content between different species, different varieties and different individuals within a species, variety or strain, (d) the effect of air drying or oven drying upon coumarin content, (e) the stage at which parent breeding material may be best selected.

Obermayer (8) was the first to develop a quantitative chemical test for coumarin in sweet clover. As a practical method for plant selection work, this test has been criticized by Ufer (10) on the grounds that it is not adapted to large-scale determinations.

Ufer (10) devised a qualitative test for coumarin in sweet clover, by means of which he has been able to test a large number of individual plants in a comparatively short time. Details of the test are not available. His selections were based upon the coumarin content of the first leaves of the sweet clover seedlings, which, he claims, possess a higher coumarin content than later leaves. He found, however, that some plants which showed a very low coumarin content in the first year showed appreciable amounts in the second year of growth. His results show the general trend of coumarin content in biennial sweet clover plants to be low in early growth stages of the first year, high in the fall of the first year, low in early growth stages of the second year and high in mature stages. The individuals that showed a relatively low coumarin content in the qualitative test were later tested quantitatively, by a modification of the Obermayer method. Coumarin-free selections from *M. albus* and *M. dentatus* were selected but failed to flower and died in the second year.

Kanevskaja and Fedrova (5) developed a quantitative test for the determination of coumarin and melilotic acid, based upon a differential ether extraction and subsequent gravimetric determination of both constituents. They report the results of analyses made on the leaves and on the stems of sweet clover plants.

Suvorov (9) states that a number of selections have been made from *M. albus*, *M. officinalis*, *M. wolgicus* and *M. dentatus*, which show as little as 0.05% coumarin. He also states that the Alpha variety showed a coumarin content of only 0.44%, as compared with 1.0% for the common type of biennial white blossom sweet clover.

Chelchinskaya and Bordunova (1) report results of investigations into the coumarin content of sweet clover, using a qualitative test as a basis of selection, while a modified form of Obermayer's procedure was used for quantitative determinations on air-dried material. They found the coumarin content of the stem to be markedly lower than that of the leaf and the coumarin content of both stem and leaf to vary greatly at different stages of growth, being highest at the flowering stage, and after this decreasing to a minimum at full maturity.

Duncan and Dustman (4) suggest a modification of Obermayer's procedure, using a steam distillation method of plant extraction for sorting out sweet clover plants of various coumarin contents.

Kirk (6) compared various types of sweet clover with respect to coumarin content, using Obermayer's method. The samples were taken at time of cutting for hay and were dried to constant weight in the laboratory before analyses were made.

Clayton (2) devised a qualitative color test for coumarin and melilotic acid in sweet clover. This test is based upon the coupling of coumarin with diazotized *p*-nitraniline in alkaline solution to give a crimson coloration.

Clayton and Larmour (3) have shown this test to be capable of quantitative interpretation with pure coumarin and melilotic acid solutions, and that a comparative, if not absolutely quantitative, determination of coumarin could be made upon plant extracts.

Experimental Procedure

The requirements of the colorimetric method of coumarin determination (Clayton and Larmour (3)) which has been used throughout these investigations are such that only a small amount of material is needed for testing. Thus uniformity to avoid errors in sampling is essential. During the earlier stages of the work one or two complete stems were taken, for each test, from individual plants. The same plants were used throughout the season. While this procedure insured that any variations in coumarin content obtained would be due to differences in stage of growth as between different times of sampling, it soon became evident that the constant pruning of the plants encouraged second growth and consequently the plants did not represent the normal growth stages. In view of this difficulty it was found necessary to use different individuals for each test. This, of course, raises the question of variation in coumarin content between different individuals within the same variety or strain. Results obtained indicate that differences do occur between individuals in certain varieties, but tests indicated a remarkable uniformity, at any particular stage of growth, among individuals within the several inbred lines used.

Preliminary tests indicated that a heavy loss in coumarin resulted from oven drying or air drying green plant material. It was decided therefore, in order to obtain the most accurate estimate of the natural coumarin content,

that the plant material should be tested in the fresh, green condition. Moisture determinations were made for each sampling and the coumarin content of each sample was expressed on the field-moisture basis. As far as possible, tests were made in duplicate. Sampling of the green material was done early in the mornings to reduce moisture loss to a minimum. No samples were taken when the plants were wet with rain or dew. Two entire stems were taken from each plant. One of these was used for moisture and the other for coumarin determination. Mature seeds were later harvested from the remaining stems of these individual plants and these, as well as a mass sample representing the entire variety or strain, were tested.

The leaves and stems of fresh green plants were examined separately. A 20-gm. sample, cut into thin sections, and 20 ml. of 50% methyl alcohol were put into a test tube marked at 50 ml. and fitted with rubber stopper and a 12-in. glass tube which served as an air condenser. The tube was immersed in a bath at 85° C. for 15 min. It was then removed, the sides were washed down with distilled water and the contents made up to 50 ml. with 50% methyl alcohol. A rubber stopper was inserted and the tube was then shaken for 1.5 hr. on a rotary shaker.

Seeds were extracted after first crushing them between layers of paper with a hammer and then grinding them in a mortar. A 0.5-gm. sample was put into the test tube, 50% methyl alcohol was added to the mark, the tube was stoppered and allowed to stand at room temperature for 12 to 16 hr. with occasional shaking, and was then shaken for 1 hr. on the rotary shaker.

In making the test on the extract, large test tubes, graduated to hold 50 cc., have been found convenient. To 5 cc. of the extract is added 5 cc. of 1.1% sodium carbonate solution and enough distilled water to half fill the tube. The contents are shaken and heated to 80° C. in a water bath for 15 minutes. To each tube is then added 5 cc. of the diazonium solution* and the tube filled to the 50 cc. mark by adding distilled water. The tubes are then stoppered, shaken and allowed to stand for at least two hours before the color comparisons are made. The volume of extract used must not contain more than 0.001 gm. of coumarin in order to be comparable with the standards used for comparison.

In order to evaluate the amount of coumarin in the material extracted, it is necessary to compare the colors obtained with those given by known concentrations of coumarin. If the color produced by the coumarin was the only color present in the extract, it would be possible to make quantitative comparisons on the colorimeter with pure coumarin solutions. However, other materials in the plant extract interfere with an accurate quantitative comparison by this method. By making up solutions from non-coumarin-containing plant extract, such as alfalfa, to which a known amount of coumarin is added, it is possible to set up a series of standards which allow comparisons to be made with reasonable accuracy. Comparisons may be made in two

*Details regarding the preparation of the diazonium solution were given in a previous paper (3, p. 93).

ways. A series of standard solutions ranging from 0–1.0% coumarin can be made up and the color of the unknown compared with these under a white light. In this way an estimate of the coumarin content can be made as being between any two of the standards, for example between 0.3 and 0.35%; or the coumarin content of the unknown may be determined by selecting the standard which corresponds most closely. Determinations are checked in all cases by means of the colorimeter. Fresh color standards for purposes of comparison are made up every four weeks; these are prepared to cover a range from 0 to 1.20%.

When only qualitative tests are made, approximately two hundred samples of either leaf or mature seed can be run in a day. The number is very much less, approximately 25, when exact quantitative tests are required.

Materials Used

The materials used in these tests were taken from the field nursery and variety test plots at Saskatoon. Thirteen varieties of *M. albus*, five varieties of *M. officinalis*, three introductions of *M. suaveolens*, one introduction of *M. wolgicus* and one of *M. dentatus* were available. In addition a large number of inbred lines of biennial white blossom sweet clover, *M. albus*, were included.

In searching for low coumarin selections, tests were made on the mature seed of several thousand additional plants obtained from various sources. In 1933 several plants of relatively low coumarin content were selected, and the progenies grown from these were available for testing in 1935.

Experimental Data

Moisture Content

Moisture determinations were made on all leaf and stem material taken for coumarin determinations. Fig. 1 represents the trend in moisture content of samples taken throughout the growing season of the second year. In the early stages, prior to flowering, the moisture content of the stem is higher than that of the leaf but it falls rapidly, being much lower than that of the leaf during the period from flowering to seed setting. The moisture content of the leaf shows a similar trend but the fall is not so rapid and it does not reach as low a point before the leaves fall from the plant. During a short period (early flowering), leaf and stem are approximately equal in moisture content.

Considering the marked variation in moisture content of the leaf and stem throughout the growing season, it was decided to calculate the actual coumarin percentages obtained on the fresh green plant material to a standard moisture basis for purposes of comparison. Since the average moisture content of the entire plant, leaves and stem, is approximately 80% during early flowering or at the stage when it would normally be harvested for hay, it appeared desirable to calculate all coumarin percentages to a basis of 80% moisture. It is evident that to express the results on a 100% dry matter basis would result in fictitiously high values for the coumarin content in the green materials and the exaggeration of variations beyond their real value.

Coumarin Content of Dried Plant Material

Anyone familiar with the odor given off from sweet clover hay during the process of curing must realize that some coumarin is lost through volatilization. Exact data on the extent of this loss have not been available. During these tests an attempt was made to determine the effect of air drying, as in haymaking, and of oven drying upon the coumarin content of the leaf and stem. In Table I are presented the results obtained from two of the several species included in this test. The plant material was harvested in the very early blossom stage. Samples of the fresh green material were tested immediately after harvesting and samples of field-cured hay and of material dried in the oven at 175° F., for two hours, were also tested.

TABLE I

Comparison of coumarin content of green, air-dried and oven-dried sweet clover

(All results calculated to basis of 100% dry matter)

Species	Green material	Air-dried in field	Oven-dried
M. albus			
Leaf	1.53	0.75	0.46
Stem	0.33	0.79	0.03
M. officinalis			
Leaf	0.96	0.54	0.16
Stem	0.28	0.71	0.07

These data indicate that much of the coumarin is lost from the stem and leaf of oven-dried material. This is to be expected since coumarin is readily volatile at the temperatures used. The data obtained from the field-cured

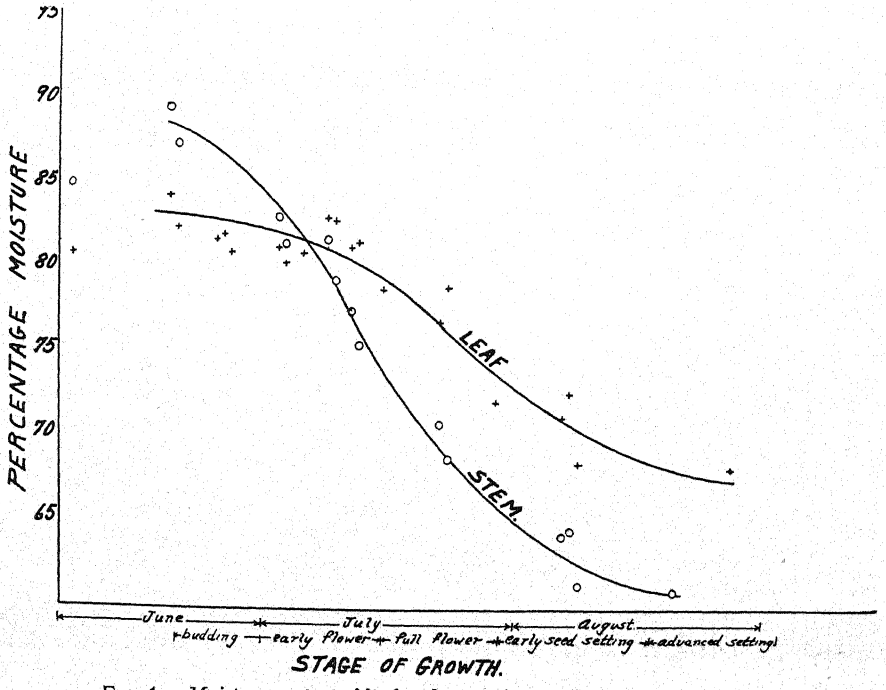

Fig. 1. *Moisture content of leaf and stem of sweet clover during the second year.*

samples is very interesting. On the dry-weight basis they show a distinct loss of coumarin from the leaves of the plant but also a marked accumulation of coumarin in the stems. This cannot be explained solely upon the basis of dehydration since the coumarin content is raised to a point beyond that shown by the stems of the fresh green material. It appears, therefore, that there is a translocation of coumarin to the stem from the leaf, or that coumarin is formed in the stem during the curing process. In view of these data it was decided that results more indicative of the actual coumarin content of the plant could be obtained by basing the test for coumarin upon the fresh, green, plant material than upon air-dried or oven-dried leaf and stem.

Variations in Coumarin Content During the Growing Season of the Second Year of Growth

The results of this phase of the work are based upon tests made, at various stages of growth, on ten lines of white blossom, biennial sweet clover which had been subjected to inbreeding and selection for at least three successive generations. This material was selected because of the apparent uniformity of type within the lines. Tests showed that there was relatively little variation in coumarin content between individuals within any of these inbred lines.

Tests for coumarin content were made on each of these lines at frequent intervals throughout the growing season. An effort was made to test samples from all lines at approximately the same stages of growth. The tests were started about thirty days prior to the time when the first buds became visible and were continued up to the time when the leaves fell and the plant matured seed. A single plant was taken from each line, for testing, at each stage of growth. Error due to individual variation, within a line, was reduced to a

TABLE II

Coumarin content (%) of leaf, stem and seed of 10 inbred lines of Arctic sweet clover (2nd year)

Plant No.	Stage of growth of vegetative material														Seed
	4-week to bud stage		2-week to bud stage		Bud stage		Early flowering		Medium flowering		Late flowering		Seed setting		Ripe seed
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	
1	0.23	0.06	0.20	0.12	0.17	0.07	0.08	0.02	0.11	0.04	0.03	0.01	–	0.01	0.40
2	0.34	0.16	0.19	0.10	0.18	0.07	0.08	0.02	0.15	0.02	0.02	0.01	–	0.01	0.40
3	0.43	0.09	0.19	0.12	0.16	0.05	0.12	0.03	0.15	0.02	0.04	0.01	–	0.01	0.47
4	0.30	0.09	0.19	0.10	0.21	0.07	0.14	0.02	0.17	0.02	0.09	0.01	–	0.01	0.66
5	0.25	0.05	0.26	0.10	0.19	0.05	0.12	0.02	0.12	0.02	0.09	0.01	–	0.01	0.57
6	0.29	0.22	0.23	0.11	0.17	0.05	0.21	0.03	0.17	0.02	0.08	0.02	–	0.01	0.45
7	0.34	0.17	0.23	0.10	0.28	0.07	0.28	0.04	0.25	0.06	0.14	0.02	–	0.02	0.47
8	0.36	0.16	0.17	0.11	0.18	0.07	0.20	0.03	0.17	0.02	0.06	0.01	–	0.02	0.43
9	0.22	0.07	0.14	–	0.18	0.05	0.14	0.02	0.15	0.02	0.03	0.01	–	0.01	0.39
10	0.19	0.07	0.13	0.03	0.16	0.06	0.08	0.02	0.13	0.02	0.07	0.01	–	0.01	0.46
Mean	0.30	0.11	0.19	0.09	0.19	0.06	0.15	0.03	0.16	0.03	0.07	0.01	–	0.01	0.47

minimum owing to the relatively high degree of uniformity within the various lines. All coumarin percentages obtained on the green material, both leaf and stem, were calculated to an 80% moisture basis. The coumarin content of the seed is given as actually determined.

In Table II are given the results obtained from each line and also the mean results for all of the lines included in the test. The general trend in coumarin content of the leaf and stem throughout the growing season is presented graphically in Fig. 2. From these data it may be observed that there is

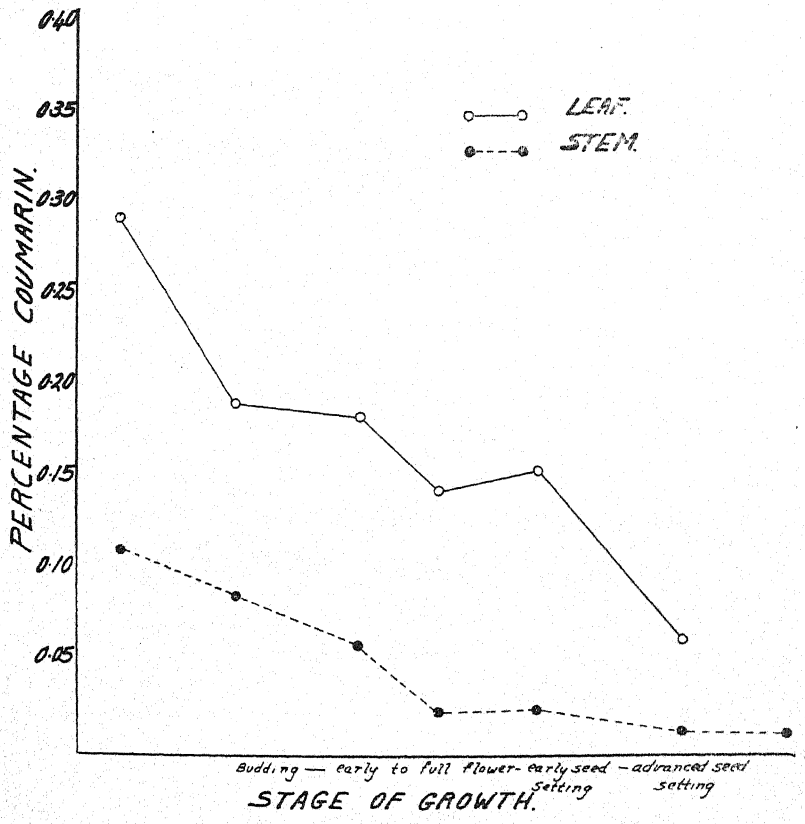

FIG. 2. *Mean coumarin content of leaf and stem of 10 inbred lines of biennial white blossom sweet clover M. albus.*

little variation between the lines with respect to the coumarin content of the stem material. They show a gradual falling off in coumarin content from before budding to the early flowering stage. From that point the fall is more rapid but there is a slight tendency to rise again at the early seed setting stage, which is again followed by a gradual decrease to an almost negligible amount at maturity. The coumarin content of the leaves shows greater variations between the different lines in that they do not all follow the same trend throughout the growing season. Fig. 3 shows the mean trend in coumarin

content of the leaf for all ten lines. Three distinct trends were obtained i the ten lines, however, and these are illustrated in Fig. 3. Type I shows rapid falling in the coumarin content from four weeks prior to budding unt

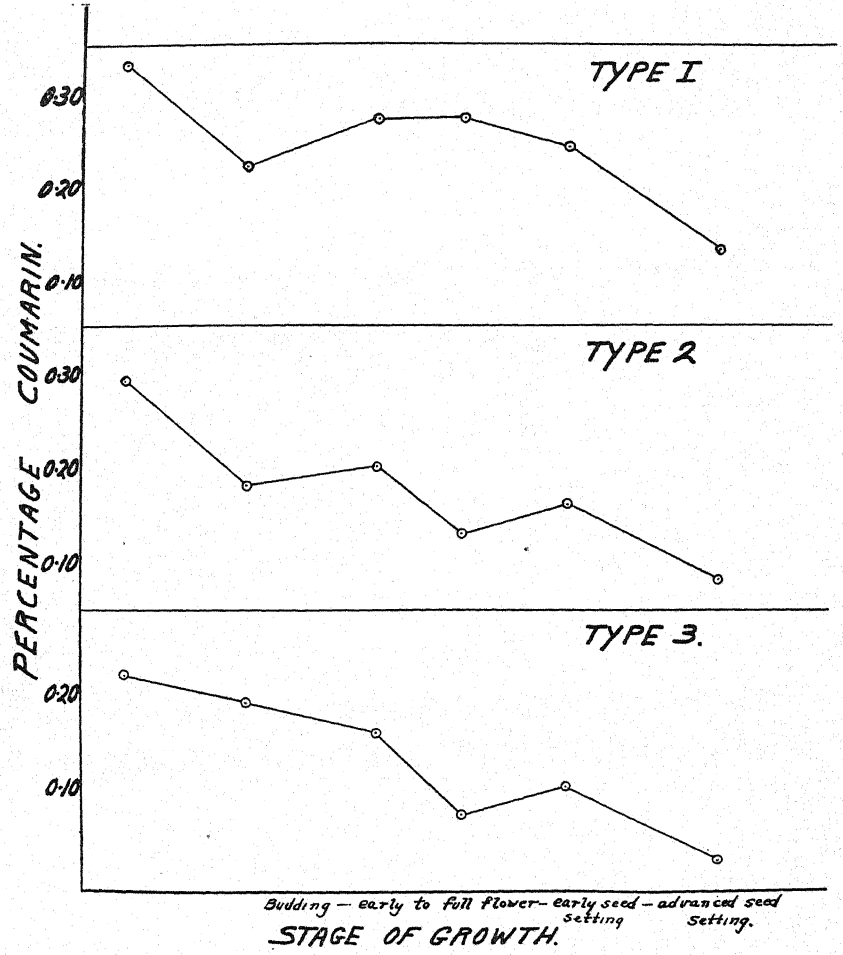

FIG. 3. *Coumarin content of leaf material of inbred lines of biennial white blossom sweet clover, M. albus.*

the budding stage. There is a gradual rise from budding to fall flowering and from this point a fairly rapid decrease to maturity. The rise in content during flowering does not reach as high a point as it attained in the early stage.

Type II shows a similar rapid falling off until the bud stage and a corresponding, although less pronounced, rise from bud to early flowering. This is followed by a very marked decrease during flowering and another increase during early seed setting, followed by a gradual decrease to maturity.

Type III shows a gradual decrease in coumarin to the early flower stage, then a rapid fall to full flower, followed by an increase during early seed-setting and finally a decrease from this point to maturity.

An analysis of these data discloses two tendencies common to all three types. First, the coumarin is higher during the period prior to budding than at any later stage. Secondly, after seed setting has begun there is a rapid falling off in coumarin content. The most common trend is represented by Type II.

It is of some interest that the rapid falling off in coumarin content during flowering corresponds to the period when the coumarin-like odor of sweet clover is most noticeable and also when the moisture content begins to diminish rapidly (See Fig. 1). It is possible that considerable coumarin is lost through volatilization, either directly or by being transformed into melilotic acid or melilotic anhydride, during this period. It should be noted that the changes in coumarin content are very great within short periods of time and that, consequently, variations in time of testing may lead to different results. No data were obtained before the plants had made eight or ten inches of growth in the spring of the second year, and consequently there are no data to show the trend during the period prior to 30 days before buds were visible. Such data will be obtained during 1936.

Comparison of Variations in Coumarin Content During the First and Second Years of Growth

Tests were made on the leaves of a number of varieties of different species, at various times throughout the first year of growth and on both the leaves and stems at different stages of growth in the second year. Seeds from this material were also tested. Tests were made on the first-year material as follows: the four-leaf stage, and 46, 74 and 94 days after planting. In the second year, tests were made at the early budding, early flowering, early seed-setting and advanced seed-setting stages. Data from some of these tests are presented in Tables III and IV.

Data obtained from tests made on material during the first year of growth show no significant differences between the various species and varieties tested. All appear to follow one general trend. Beginning with the young seedlings, four-leaf stage, the coumarin content in each case is low and in most cases it increases rather rapidly, reaching a maximum when the plants are about 10 to 12 in. high (in this case 46 days after the four-leaf stage), and then falls rather rapidly to the end of the growing season. Data obtained from tests made on the same material in the second year of growth reveal some rather striking differences between the various varieties and species used. The general trend, however, is a rise in coumarin content to a maximum during the flowering stage, followed by a steady decrease to maturity. In general the trend is similar to that obtained for the inbred lines of white blossom sweet clover. However, there were relatively few tests made on this material throughout the growing season, and consequently some of the variations in coumarin content, which take place rather rapidly at certain times, have not been recorded. The result is a tendency toward a unimodal curve with the highest point during the flowering period.

TABLE III

COUMARIN CONTENT OF LEAF MATERIAL OF SOME *Melilotus* SPECIES (1ST YEAR)

Species	4-leaf stage	Days after planting		
		36	74	94
M. albus No. 1	0.48	0.45	0.24	0.22
M. albus No. 2	0.18	0.44	0.29	0.27
M. officinalis	0.37	0.51	0.23	0.20
M. suaveolens	0.24	0.44	0.30	0.27
M. dentatus (annual)	0.01	0.01	0.01	0.01*

**Less than 0.01 in each case.*

TABLE IV

COUMARIN CONTENT OF LEAF MATERIAL AND SEEDS OF SOME *Melilotus* SPECIES (2ND YEAR)

Species	Leaf material				
	Early budding	Early flowering	Flowering with seed-setting	Advanced seed-setting	Mature seed
M. albus No. 1	0.39	0.40	0.23	0.06	0.79
M. albus No. 2	0.18	0.23	0.15	0.09	0.37
M. officinalis	0.30	0.18	—	0.07	0.74
M. suaveolens	0.32	0.41	0.35	0.15	0.43
M. dentatus (annual)	0.01	0.01	0.01	0.01	0.05

The annual form of *Melilotus dentatus* included in these tests may be, fo all practical purposes, regarded as coumarin-free. The tests made on th leaf and stem material show less than 0.01% coumarin and the mature see 0.05%. It is worthy of note also that the coumarin content of the Alph variety of biennial white blossom sweet clover is considerably lower than tha of the other varieties of *M. albus* tested.

There appears to be no marked difference in coumarin content between th two most widely grown species, *M. albus* and *M. officinalis*. Variation between the different varieties within these species are fairly large.

During the testing of these various types and varieties of sweet clover i was noted that there appeared to be a marked relation between the coumarin content and the color of the foliage. Sweet clover presents marked variations in color of foliage, which ranges from light yellowish green to dark, almost bluish, green. The plants with dark colored foliage invariably contained a higher coumarin content than those possessing the lighter green color.

COMPARISON OF COUMARIN CONTENT IN LEAF AND MATURE SEED

In view of the fact that rapid changes in the coumarin content of the leaf and stem take place during the growing season, it is essential that tests be conducted at the same stage of growth for all individuals, if comparative data of value are to be obtained. It appears desirable therefore to seek some phase

in the life history of the plant, in which the coumarin content is more constant, upon which to base coumarin determinations. Thus an effort was made to determine to what extent, if any, the coumarin content of the leaf is reflected in that of the mature seed. The coumarin content of the leaf at full flowering stage, correlated with that of the mature seed for the twenty different varieties and species tested, showed a fairly significant correlation.

Breeding Work

In the winter of 1933, seeds from several thousand single plant selections were tested for coumarin content. Five selections were made which contain between 0.10 and 0.15% coumarin. Four individuals contained more than 1.0%. The average for all plants tested was 0.57%. Progenies from the selections of lowest and highest coumarin content were planted in the field nursery in the spring of 1934. Selfed seed was harvested from the individual plants in these progenies during the fall of 1935 and has been tested for coumarin content. The frequency distribution of coumarin content of individuals is shown in Table V. The data from the low-coumarin selections

TABLE V

A COMPARISON OF COUMARIN CONTENTS OF PROGENIES GROWN FROM HIGH- AND FROM LOW-COUMARIN SELECTIONS OF WHITE BLOSSOM BIENNIAL SWEET CLOVER *(M. albus)*

Selection No.	Classes in percentage*															
	.05	.10	.15	.20	.30	.40	.50	.60	.70	.80	.90	1.0	1.05	1.10	1.2	1.3	1.4
1	–	–	–	12	50	20	1	–	–	–	–	–	–	–	–	–	–
2	–	–	1	15	32	25	5	4	–	–	–	–	–	–	–	–	–
3	–	–	11	26	24	7	2	1	–	–	–	–	–	–	–	–	–
4	–	–	–	5	17	12	7	2	–	–	–	–	–	–	–	–	–
5	–	–	3	11	21	3	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	1	2	6	4	14	4	3	6	3	–
7	–	–	–	–	–	–	1	–	2	2	–	4	1	3	14	5	–
8	–	–	–	–	–	–	–	–	1	1	2	2	3	5	11	6	2
9	–	–	–	–	–	2	10	5	8	3	3	1	1	–	–	–	–

NOTE:—*The low-coumarin selections, Nos. 1–5 inclusive, contained less than 0.15% coumarin. The high-coumarin selections, Nos. 6–9 inclusive, contained a coumarin content of 1% or higher.*

**Class figures given represent upper limits of the class range.*

show definitely that most of the population tend to be relatively low in coumarin, an appreciably large percentage of the individuals showing a coumarin content of between 0.1 and 0.2%. The data from the high-coumarin selections, on the other hand, show that the greater part of the populations are of relatively high coumarin content. The greatest frequencies are in the 0.91–1.2% coumarin classes. The coumarin content of both low- and high-coumarin progenies tends to be higher than that of the corresponding original selections which were grown the previous year. This is believed to be due to seasonal influences.

The progeny plants from the low-coumarin lines appeared perfectly normal in the field. There were no indications of lack of vigor. Without exception these plants produced seed abundantly.

These results indicate that the coumarin content of *M. albus* is determined largely by heredity, and that it may be possible to isolate lines containing relatively little coumarin, through continuous selection within inbred lines.

The other phase of the breeding work has to do with crossing the almost coumarin-free *M. dentatus* with species of recognized agricultural value. Up to the present time we have not been successful in bringing to maturity the plants presumed to be F_1 hybrids. Seeds were obtained without difficulty when *M. albus*, as the female parent, was crossed with *M. dentatus*. These seeds germinated but the young seedlings were, without exception, weak and lacking in chlorophyll. They died within two or three weeks after emergence. No seeds were obtained from the reciprocal cross.

Acknowledgments

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A FOUR-YEAR QUANTITATIVE STUDY OF NITROGEN-FIXING BACTERIA IN SOILS OF DIFFERENT FERTILIZER TREATMENT[1]

By A. G. Lochhead[2] and R. H. Thexton[3]

Abstract

Numbers of *Rhizobium trifolii*, *Rh. leguminosarum*, *Rh. meliloti* and *Azotobacter* were determined at four-week intervals throughout a four-year crop rotation in three soils which had been receiving for twenty years no fertilizer, manure, and artificial fertilizer respectively. Though relatively small differences were noted in numbers of *Rh. trifolii* in the three soils, *Rh. leguminosarum* and *Rh. meliloti* persisted in much higher numbers in the two fertilized areas than in the unfertilized soil. *Rh. trifolii*, the only species with host plant in the rotation, occurred in much greater numbers than the other species, not only during and immediately following clover, but in succeeding years when little or no decline was noted. Apart from the effect of clover on *Rh. trifolii* no significant effect of cropping was noted nor was seasonal influence important. Freezing of the soil for three months each year produced little or no effect on the numbers of *Rhizobia*.

Numbers of *Azotobacter* were consistently higher in the unfertilized soil than in the fertilized areas. A seasonal effect was noted, with maximum numbers in March and minimum numbers in July, while freezing caused no noticeable diminution in colony count. The numbers of *Azotobacter* found were in all cases low and suggest that the part played by this organism in nitrogen fixation in field soil is still obscure.

Rh. trifolii, *Azotobacter* and total numbers of bacteria by the plate method showed no relation with the productivity of the soils. Numbers of *Rh. leguminosarum* and *Rh. meliloti* showed better agreement, though only in the case of *Rh. meliloti* were relative numbers consistent with the soils in order of crop yields throughout the rotation.

Introduction

The influence of such factors as season, cropping and fertilizer treatment upon the general microflora of soils has been the subject of considerable investigation. Less attention has been paid to the effect of season and soil management practices upon specific groups of soil micro-organisms. It might be reasonably assumed, however, that studies of the influence of various factors on the numbers and activities of definite physiological groups of soil micro-organisms would contribute more to our knowledge of soil processes than analogous data on the soil microflora as a whole. It is conceivable that pronounced changes in the numbers of two or more groups of organisms, reacting differently to a given influence, may be quite unrecognized by a method of "total counts."

The nitrogen-fixing bacteria, symbiotic and non-symbiotic, are commonly regarded as groups of organisms of definite significance in the maintenance of fertility in most arable soils. Being physiologically specialized types, they might be expected to react in their own fashion to the various influences of climate, cropping and soil treatment. The persistence of the different cross-inoculation groups of nodule-forming organisms in soil is of definite import-

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ance in relation to the growth of legume crops and the need for re-inoculation while non-symbiotic fixation must be influenced by the numbers of nitrogen-fixing organisms as they react to their environment.

By far the greater number of quantitative studies of the microflora of soils have represented examinations carried out during the actual or potential crop producing period, that is, from spring to autumn. In regions such as are represented by the greater part of Canada, where the soil may remain frozen for a part of the year, often two to three months or more, it is reasonable to assume that the winter season must also be considered in attempting to form any comprehensive picture of soil microbiological processes as a whole. The present study is an attempt to follow for four consecutive years the numbers of three species of *Rhizobium* and of *Azotobacter* under definite conditions of cropping and fertilizer treatment.

Historical

Various studies of a qualitative nature have shown that certain species of nodule bacteria may remain alive in soils, apart from the host plant, for a number of years. Albrecht and Turk (1) in surveying the literature, emphasize the wide range in the period of time through which legume bacteria have been reported to survive. Little information of a quantitative nature respecting the incidence of *Rhizobium* in soils and the effect of environment on the numbers was available, however, until the development of a suitable method of enumeration. Through the application of a suitable dilution method, however, Wilson (20–24) was able to study quantitatively the legume bacteria population of soils, and indicate the value of a counting technique for studying the relations of different species of *Rhizobium* to their environment.

Examining a series of soils from October to June, Wilson (21) found in general a drop in numbers of *Rhizobium* as the winter advanced, followed by a rise in spring. Reporting studies (22, 24) on the relative numbers of different species of *Rhizobium*, he found that soils vary in their capacity to maintain different types. *Rh. trifolii* was found in general in much higher numbers than *Rh. leguminosarum* which latter species was in general more abundant than *Rh. japonicum*. Little or no significance was attached to the reaction of the soil, the moisture content, the season, or the crop on the soil. The author points out that the numerical relation of these species of *Rhizobium* in soils coincides with the hardiness of the organism in artificial cultures and also with the hardiness of the respective host plants in the field. From a special study (23) of *Rh. japonicum* the writer concluded that organisms liberated from the nodule tissue of the host crop diminish rapidly and reach minimum numbers or practical extinction in two or three years.

Walker and Brown (19) applied the Wilson method to a study of *Rh. trifolii* and *Rh. meliloti* in variously treated soils, and found that, in general, the numbers depended upon the previous cropping history of the land, and

upon fertilizer treatment. They found, however, that the condition of the soil with reference to organic matter, lime and phosphate, had a much greater influence on the numbers of organisms than the frequency of growth of the host plant. They suggest that recommendations for inoculation should be based, not only upon the cropping system, but also upon a knowledge of the soil management practices followed.

Azotobacter is an organism of wide distribution in soils. Its absence is probably due, in the majority of cases, to an unfavorable reaction, since it does not normally develop in soils of an acidity greater than pH 6.0, though Wilson and Wilson (25) point out the importance of the "soil complex," particularly the carbonate-phosphate ratio, in controlling the activity of this organism. Under laboratory conditions, response to additions of fertilizers to samples of soil has been repeatedly shown by this organism, with or without added inoculum; and the development of the soil plaque method has added to our methods of estimating fertilizer requirements. There is, however, much less information as to the numbers of this organism in soil under field conditions, and to the effect of climatic and soil management practices.

Quantitative studies have indicated that *Azotobacter* is present in soils in comparatively small numbers, generally hundreds or a few thousands per gram, though microscopic studies would indicate larger numbers (18). Our information as to the effect of environment on *Azotobacter* in the soil has been largely inference from nitrogen-fixation tests carried out in soil or solution. Since nitrogen fixation in soils is by no means the exclusive function of *Azotobacter* the occurrence of this organism is more accurately measured by more direct methods. Evidence of a more direct nature was possible by the use of the silica-gel plating medium devised by Winogradsky (26) for developing colonies of *Azotobacter* directly from soil.

Application of the silica-gel plate method to the estimation of the *Azotobacter* population of soils has been made by a number of investigators (3, 11, 14, 16, 28, 29). Counts reported have been generally quite low, ranging for the most part from none to several hundred per gram of soil, with occasional counts showing several thousand per gram. Winogradsky and Ziemięcka (28) in a study of the effect of fertilizers, noted a depression of *Azotobacter* numbers in soils receiving applications of nitrogen. Curie (3), developing an agar plate method from the silica-gel technique, reported a lower *Azotobacter* population in soils receiving additions of manure, as compared with control plots, a similar depressing effect of nitrogen being noted by Ziemięcka (29) in a study of Rothamsted soils. Enumerating *Azotobacter* by the silica-gel plate method, Vandecaveye and Anderson (16) in a study of seasonal trend, found at most a few hundred colonies per gram in spring and fall, with organisms absent from gram portions in midsummer. Turk (14) likewise found small numbers in Michigan soils. In a recent study of Swiss soils, Stöckli (11) reported counts of a few hundreds or thousands, the numbers tending to rise with increase in pH value and amount of available phosphoric acid.

Methods

Determinations were made of the numbers of *Rhizobium trifolii*, *Rh. leguminosarum* and *Rh. meliloti* at four-week intervals from November, 1931, until October, 1935, in three soils of different fertilizer treatment supporting a rotation of mangels, oats, clover and timothy. The tests were commenced following the clover. At the start of the experiment the three soil areas had received for 20 years the following fertilizer treatments:

Soil N—No fertilizer.

Soil X—15 tons manure per acre, applied to mangels.

Soil Y—100 lb. nitrate of soda, 300 lb. superphosphate, 75 lb. muriate of potash to mangels, 100 lb. nitrate of soda to oats, clover and timothy.

The three soils, at the start, contained respectively 0.113%, 0.162% and 0.126% nitrogen, and all showed a reaction slightly more alkaline than pH 7.0. The productivity for 24 years and for the period of the experiment is indicated in Table I.

TABLE I

CROP YIELDS OF SOILS

—	Timothy, tons per acre	Mangels, tons per acre	Oats, bu. per acre	Clover, tons per acre
Soil N, av. 24 years	2.04	8.22	45.7	2.08
av. 1931-1935	1.82	2.15	47.1	1.49
Soil X, av. 24 years	3.11	22.46	59.9	3.79
av. 1931–1935	2.83	22.90	64.9	3.56
Soil Y, av. 24 years	2.67	20.74	54.6	3.45
av. 1931–1935	2.31	21.03	60.6	2.76

Samples were taken at a depth of two to three inches, normally from the sides of a pit approximately three feet square. When the soil was frozen it was sampled by removing about one square yard of the surface to a depth of two inches, with a pick, and chipping off portions from the two- to three-inch level. The samples, after thawing, if necessary, were passed through a 3-mm. sieve, mixed, and dilutions prepared by adding 20 gm. to 200 cc. sterile water.

The method of determining the numbers of *Rhizobium* was essentially that used by Wilson (20). Small enamel crocks of 250 cc. capacity containing a mixture of equal quantities of soil and sand were sterilized and inoculated in duplicate with 1 cc. of a series of dilutions (1–10, 1–50, 1–100, 1–500, 1–1,000, up to 1–10,000,000). After moistening with sterile water, the crocks were covered and incubated at 28° C. for one week. Seed of alfalfa, red clover and vetch, after being sterilized with mercuric chloride were planted in appropriate pots corresponding to the dilutions found necessary to insure absence of inoculation with the highest dilution. After two to three weeks in the greenhouse, depending upon the growth, the roots of the seedlings were examined for nodules, and the numbers of the three species of *Rhizobium* estimated from their capacity for nodule production on the respective host plants.

The numbers of *Azotobacter* were estimated by an adaptation of the agar plate method proposed by Curie (3). The medium used contained, per litre, mannite, 15.0 gm.; dipotassium hydrogen phosphate, 0.5 gm.; magnesium sulphate, 0.2 gm.; sodium chloride, 0.2 gm.; calcium carbonate, 1.0 gm.; manganese sulphate and ferric chloride, 1 drop of 5% solution each, and agar 1.5 gm. The medium was poured into ordinary Petri dishes (25 cc. each) and allowed to harden. Upon the surface was scattered uniformly 0.3 gm. of the soil, passed through a 20-mesh but retained on a 40-mesh sieve. For each sample of soil 10 plates were inoculated, incubated at 28° C. for three to four days, and *Azotobacter* colonies counted. Soils which were too moist to handle were partially air-dried before inoculating, moisture determinations being made on the soil so used as well as on the samples coming from the field. For the first year of the experiment *Azotobacter* counts were also made by the dilution method. Flasks of 200 cc. capacity, containing 25 cc. of a mannite solution similar to the above-mentioned medium, were inoculated, in duplicate, with decreasing quantities of soil. Flasks were incubated at 28° C. and examined microscopically for *Azotobacter*. During the first year, determinations were made of the so-called "nitrogen-fixing capacity" of the soils. Four 500 cc. Erlenmeyer flasks, containing 100 cc. of the above mannite solution, were inoculated with 5 gm. of soil. Two flasks were then sterilized as controls and two flasks incubated at 28° C. for 14 days, and total nitrogen determined by the usual Kjeldahl method.

During the last three years of the experiment determinations were made of total numbers of bacteria and actinomyces in the soils. Plate counts were made on the synthetic agar medium described by Thornton (12), incubated at 28° C. for 10 days.

Results and Discussion

The data from 55 consecutive analyses for the various organisms on the three soils, extending from November 10, 1931, until October 11, 1935, are shown graphically in Figs. 1 to 3, while yearly summaries (November to October) are given in Table II.

Rhizobium

The results indicate that the three species of *Rhizobium* studied not only varied in their actual abundance, but also showed relative differences varying with the soil. Thus in the case of *Rh. trifolii* (Fig. 1 and Table II) there is comparatively little difference in the capacity of the three soils to support this organism. In the first year following the crop of clover the manured area gave higher average counts, but in the following years less difference was noted between the soils. In the case of *Rh. leguminosarum* it is plain that Soil N (no fertilizer) supports a much lower population than either of the soils receiving fertilizer. Thus Soil X (manure) contained approximately 21 times as many, and Soil Y (artificial fertilizer) 25 times as many organisms as Soil N, considering averages for the four-year period. Similarly, with *Rh. meliloti* the two fertilized areas, X and Y, were found to support respectively 10 and 6 times as many organisms for the four-year period as Soil N.

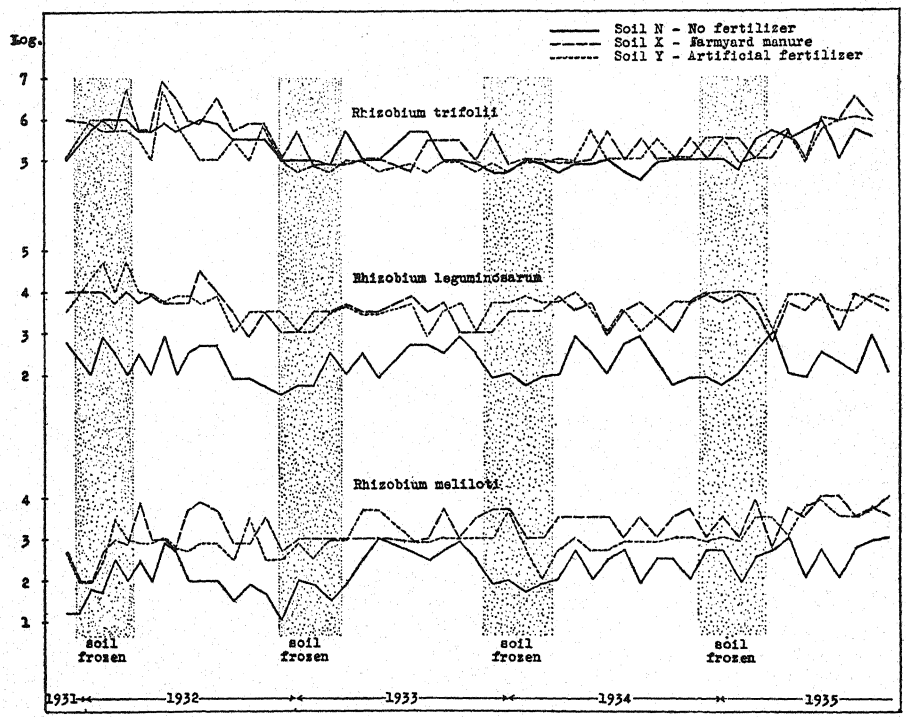

FIG. 1. *Numbers of Rhizobium trifolii, Rh. leguminosarum and Rh. meliloti in soils of different fertilizer treatment.*

TABLE II

SUMMARY OF COUNTS OF BACTERIA IN SOILS AT FOUR-WEEK INTERVALS FROM NOVEMBER, 1931 TO OCTOBER, 1935

Soil and year	Bacteria per gram of soil (log. average)				
	Rh. trifolii	*Rh. leguminosarum*	*Rh. meliloti*	*Azotobacter*	Total count
Soil N (No fertilizer)					
First year after clover	379,000	235	130	100	—
Second year after clover	161,000	155	155	113	13,700,000
Third year after clover	67,000	170	165	172	30,100,000
Clover crop	226,000	155	295	130	23,400,000
Average for 4 years	**174,000**	**175**	**176**	**123**	**21,500,000**
Average when soil frozen	**186,000**	**140**	**120**	**134**	**25,000,000**
Soil X (Manure)					
First year after clover	1,119,000	6,320	990	37	—
Second year after clover	158,000	2,690	1,160	61	22,400,000
Third year after clover	131,000	2,400	2,330	64	31,100,000
Clover crop	345,000	3,710	3,160	79	32,900,000
Average for 4 years	**310,000**	**3,600**	**1,690**	**55**	**28,300,000**
Average when soil frozen	**230,000**	**3,650**	**1,360**	**58**	**33,500,000**
Soil Y (Artificial fertilizer)					
First year after clover	370,000	7,360	590	59	—
Second year after clover	90,000	2,540	910	56	27,600,000
Third year after clover	104,000	3,360	720	67	33,400,000
Clover crop	293,000	5,370	2,810	76	31,200,000
Average for 4 years	**184,000**	**4,340**	**1,000**	**64**	**30,800,000**
Average when soil frozen	**156,000**	**5,890**	**890**	**69**	**39,200,000**

Rhizobium trifolii was present in all three soils in much greater numbers than the other legume bacteria studied. As it was the only species with its host plant in the crop rotation, its greater abundance during the year following clover might well be expected. After the first year further decline was slight or absent, however, the numbers remaining at a comparatively high level and suggesting that the three soils are capable of supporting a much higher population of *Rh. trifolii* than of *Rh. leguminosarum* or *Rh. meliloti*. This is in agreement with the results of Wilson (24), who reported finding *Rh. trifolii*, *Rh. leguminosarum* and *Rh. japonicum* in rather definite numerical order.

Table III shows the average relative numbers for the second and third years following clover, with comparative data calculated from Wilson's findings. In the fertilized areas, X and Y, ratios are of the same order as those reported by Wilson. Soil N, which had received no fertilizer for over 20 years, was relatively much less suited to *Rh. leguminosarum* and *Rh. meliloti*.

TABLE III

RELATIVE NUMBERS OF SPECIES OF *Rhizobium*

—	*Rh. trifolii*	*Rh. leguminosarum*	*Rh. meliloti*	*Rh. japonicum*
Soil N (no fertilizer)	1,000	1.6	1.6	—
Soil X (manure)	1,000	18	12	—
Soil Y (artificial)	1,000	30	8	—
Wilson (average of 55 samples)	1,000	14	—	0.17

The effect of its symbiont on the numbers of *Rh. trifolii* was noted during the year following clover (1932), the crop being ploughed under that spring preparatory to sowing timothy. The numbers remained more or less constant the second and third years (1933 and 1934) though in the spring of 1934 clover was sown with oats. During that year the plants did not develop to more than two or three inches in height, and no noticeable effect was found on the average numbers of nodule organisms in the soil. Only with the spring and summer of 1935, when the clover crop developed, was the presence of the symbiont reflected by a general rise in numbers of *Rh. trifolii*.

Apart from the effect of the recurrent growth of clover on *Rh. trifolii*, the study did not indicate any significant effect of the crops upon the numbers of the nodule organisms studied, nor did the moisture content of the soil or the season of the year appear to be of significance. Previous studies by one of us (6) on the effect of frost on the general microflora of soil had shown that after more than three months' freezing bacterial numbers remained at normal levels. Data from the present work (see Table II) indicate that the legume nodule bacteria in the soils studied are to be regarded as cold tolerant, *i.e.*, able to withstand the prolonged period of continuous freezing with relatively little or no diminution in numbers. This ability to tolerate continued frost is believed to be an important factor in the maintenance of successful legume cultivation in regions with severe winters.

Azotobacter

The data from the individual *Azotobacter* determinations are shown graphically in Fig. 2, while summaries for the successive years and average monthly values are presented in Tables II and V respectively. It is noted that Soil N,

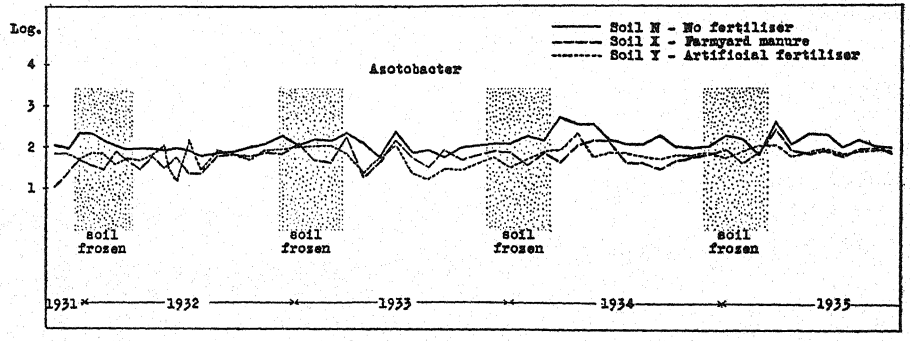

FIG. 2. *Numbers of Azotobacter in soils of different fertilizer treatment.*

without fertilizer for more than 20 years, and giving much lower crop yields than Soils X and Y, showed consistently the highest numbers of *Azotobacter*. The depressing effect of farmyard manure and nitrate fertilizer respectively on the numbers of *Azotobacter* is in agreement with the observations of Winogradsky and Ziemięcka (28), Batchelor and Curie (2), Curie (3), Gainey and Sewell (4), and Ziemięcka (29) who noted a reduction in the growth of *Azotobacter* from the application of nitrogenous fertilizers.

During the first year of the study, November, 1931, to October, 1932, parallel determinations were made of *Azotobacter* by the dilution method and of the nitrogen-fixing capacity of the soils in solution. The results, summarized in Table IV, show a general agreement between the dilution and the plate methods, though the absolute numbers found were higher with the former. Nitrogen fixation tests, however, indicated little or no difference between the soils, and are regarded as distinctly inferior to tests of the *Azotobacter* population, as determinable by silica-gel or agar plates and soil plaques in evaluating the *Azotobacter* flora of a series of soils.

TABLE IV

COMPARISON OF PLATE COUNT, DILUTION COUNT AND NITROGEN-FIXATION TESTS

Average, 16 determinations, November, 1931–October, 1932	Soil N (no fertilizer)	Soil X (manure)	Soil Y (mineral fertilizer)
Plate count, colonies per gram	100	37	59
Plate count, ratio	**100**	**37**	**59**
Dilution count, per gram	304	84	175
Dilution count, ratio	**100**	**28**	**58**
Nitrogen-fixation, mg. per 100 cc.	11.93	11.78	12.80
Nitrogen-fixation, ratio	**100**	**99**	**107**

A certain seasonal fluctuation in numbers of *Azotobacter* may be observed from Table V, giving the four-year monthly averages from the plate counts. In all soils, minimum numbers were found in July, in line with results reported by Vandecaveye and Anderson (16). Maximum numbers, however, were in

TABLE V

Azotobacter COUNTS—MONTHLY AVERAGES, 4 YEARS

Month	Colonies per gram dry soil		
	Soil N (no fertilizer)	Soil X (manure)	Soil Y (mineral fertilizer)
January	148	75	63
February	127	49	77
March	247 (max.)	99 (max.)	80 (max.)
April	145	56	62
May	133	58	69
June	133	72	69
July	80 (min.)	41 (min.)	41 (min.)
August	97	51	51
September	93	66	57
October	98	55	53
November	108	63	72
December	135	52	66

all cases found in March, in which month samples were all taken in the latter half, after the first thawing had occurred. This rise in numbers immediately following the period of frost was noted by one of us (6) in a study of the general bacterial soil flora. The data do not indicate any depressing effect of cold, however, the average counts being well maintained during the winter months.

One of the most striking features of the present study, as well as those of other workers, is the generally low number of *Azotobacter* in soils as indicated by any cultural method of enumeration. When one finds a few hundreds or at most a few thousands in a gram of soil which may harbor a bacterial population of one hundred million in addition to numerous fungi, protozoa, etc., one may reasonably doubt the importance of *Azotobacter* as an agent of nitrogen fixation under field conditions. The lack of exact knowledge of the role of this organism in the field has been emphasized by such workers as Waksman (17) and Winogradsky (27). Data from physiological studies of *Azotobacter* in pure culture, and from nitrogen-fixing capacity tests under artificial conditions do not appear to provide an answer to the question.

It is possible that our counting methods do not give a reasonable estimate of *Azotobacter* numbers in soil, since microscopic observations suggest, though they do not prove, a higher population. Thus Smith (10) found strains of *Azotobacter* which do not utilize mannite and would therefore be missed with media depending upon that carbohydrate, while van Niel (15) found failure of certain soils to give *Azotobacter* growth in an elective culture medium to be due to deficiency of molybdenum. Control tests did not indicate that these particular factors were operating in the present experiment. The recently

reported findings of filterable forms of *Azotobacter* in soil by Norogrudsky and Messineva (8, 9) suggest further the possible presence of this organism in numbers not indicated by present counting methods. Further progress in methods for determining the real *Azotobacter* population of soils would greatly help in forming an estimate of the actual role played by the organism in nitrogen fixation in field soils. Furthermore, the presence in soil of numerous other aerobic organisms to which capacity for nitrogen fixation has been ascribed, and more particularly, the possible importance of anaerobic bacteria as agents of nitrogen fixation under practical conditions confirm the belief that the part played by *Azotobacter* is an open question.

Total Bacteria Counts

Data from the total counts of bacteria and actinomyces, seen in Fig. 3 and Table II, do not indicate marked differences between the soils studied. Somewhat higher average counts were obtained from the two fertilized areas,

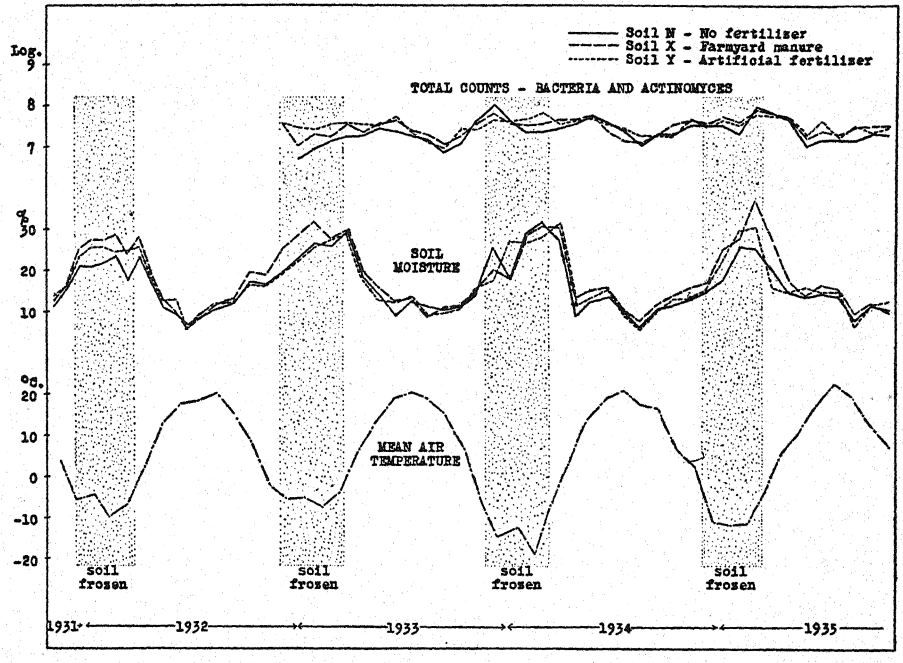

FIG. 3. *Total counts of bacteria and actinomyces, soil moisture and air temperature.*

X and Y, than from the unfertilized area, with numbers well maintained when the soil was frozen. As noted in other investigations with Canadian soils (5, 6, 7, 13), a certain seasonal trend was apparent, with generally lower numbers from July to September following higher numbers in spring. The data indicate, however, that for estimating the relative abundance of specific physiological groups of organisms in soil, such as nitrogen-fixing types, the total count method is unreliable. Its application to problems of soil fertility, then, must be considered as very limited.

Micro-organisms and Crop Yields

A comparison between the relative productivity of the three areas studied, and the average values for the bacterial counts are given in Table VI. Relative average yields for the different crops during the period of the test are given as well as relative counts (logarithms) for the various groups of micro-organisms.

TABLE VI

RELATIVE YIELDS AND NUMBERS OF BACTERIA

Crop	Soil area	Relative values					
		Crop yield	*Rh. trifolii*	*Rh. leguminosarum*	*Rh. meliloti*	*Azotobacter*	Total count
Timothy	N	100	100	100	100	100	—
	X	164	109	161	153	77	—
	Y	134	99	167	142	87	—
Mangels	N	100	100	100	100	100	100
	X	1,070	100	157	140	87	103
	Y	950	95	156	135	85	104
Oats	N	100	100	100	100	100	100
	X	138	106	152	152	81	100
	Y	129	104	159	149	83	101
Clover	N	100	100	100	100	100	100
	X	239	104	163	142	90	102
	Y	185	102	170	139	88	101

The relative productivity varies with the crop, the difference between the unfertilized and fertilized areas being most emphasized with mangels and less with the other crops. Although this pronounced difference with the hoed crop is not reflected in the bacterial counts, yet the relation of productivity to micro-population is of interest.

With total count, *Azotobacter* count, and *Rh. trifolii* count, no relation to productivity can be noted. The numbers of *Rh. leguminosarum* and *Rh. meliloti* show distinctly higher values when the fertilized areas are compared with the less productive area, N. Only in the case of *Rh. meliloti*, however, do the numbers found coincide in order with the order of productivity of all three soils for all four crops. It is suggested from the findings that study of the abundance of certain species shows greater promise of providing a reliable index of soil fertility than the numbers of micro-organisms as a whole.

Acknowledgment

The authors are indebted to the Field Husbandry Division, Central Experimental Farm, for co-operation throughout the course of the experiment and to the Division of Chemistry for the nitrogen determinations.

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THERMAL DEATH POINT OF CERTAIN WEED SEEDS[1]

By Clarence Yardley Hopkins[2]

Abstract

A series of weed seeds comprising seven species and representing five families was studied to determine the effect of heat on subsequent germination. Lethal temperatures for 15 minutes' heating varied from 85° to 105 ° C.

The results indicate that there is a critical temperature below which moderate periods of heating have little effect on viability. At higher temperatures the germinating power falls off rapidly.

Introduction

During a study of the utilization of refuse grain screenings, it occurred to the writer that a simple process for killing the weed seeds in screenings would be of practical value.

It is well known that the application of sufficient heat will destroy the germinating power of seeds. The effective temperatures and duration of heating have been determined for a number of seeds of crop plants. The present work was undertaken in order to determine the corresponding lethal conditions for some of the most common weed seeds found in refuse screenings.

A careful study of the effect of heat on radish seeds was made by Waggoner in 1917 (6). Other investigators have worked with cotton seed (3, 4) and with wheat (1, 8). The subject is reviewed and a bibliography appended in references 6 and 8.

It has been shown by these authors that the moisture content of the seed during the period of heating is an important factor in determining the lethal temperature. This condition depends not only on the initial moisture content of the seed, but also on the design of the heating chamber, *i.e.*, whether or not it allows free escape of moisture. Seeds which are very dry or are exposed to the drying effect of much warm air during the treatment will resist unusually high temperatures.

Experimental

Materials and Methods

The method of heat-treating the seeds was designed with the above considerations in mind. Furthermore, the conditions of the experiments were such that they could be easily duplicated in large-scale operation.

Most of the seeds were obtained from the weed nursery at the University of Saskatchewan through the kindness of T. K. Pavlychenko. A few samples were selected from refuse screenings taken from grain at Fort William.

The seeds were conditioned at 50% relative humidity at 25° C., so that all were comparable with respect to moisture content. The heating was carried out in narrow brass tubes, closed at the lower end and stoppered with a plug

[1] *Manuscript received February 15, 1936.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

[2] *Chemist.*

of absorbent cotton. The tubes were immersed nearly to the top in a well-stirred oil bath with thermostatic control. Temperature readings were taken from a thermometer inside a similar tube. The seeds were treated in lots of such a size that the tube was filled to a depth of about one inch. The sample was then about two inches below the surface level of the bath. Following the heat treatment, the sample was poured into a bottle and allowed to cool before the bottle was stoppered.

Germination tests were carried out at the laboratory of the Seed Branch, Dominion Department of Agriculture, through the courtesy of the staff of that department.

Experimental Results

The relation of the temperature of heating to subsequent germination of some of the common weed seeds is shown in Figs. 1 and 2. The period of heating in each case was 15 minutes.

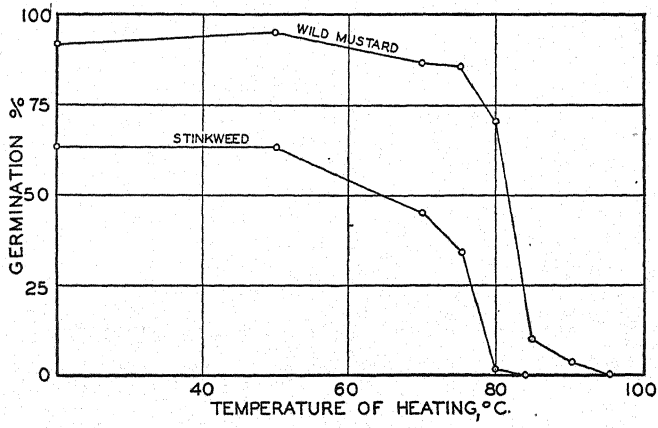

FIG. 1. *Effect of 15 minutes' heating on subsequent germination (Cruciferae).*

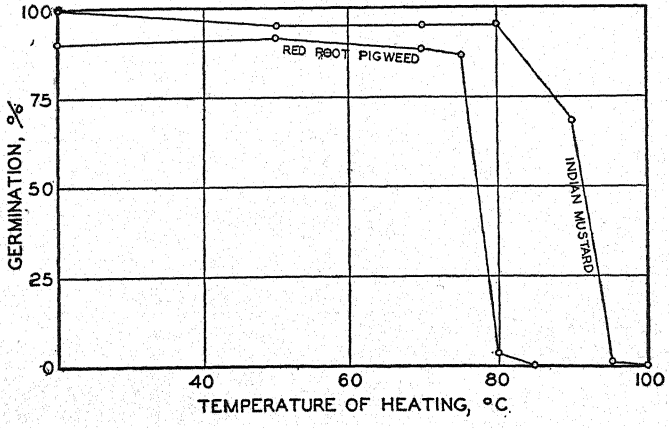

FIG. 2. *Effect of 15 minutes' heating on subsequent germination. (Cruciferae and Amaranthaceae).*

Figs. 3 and 4 show the temperature-germination curves for lambs' quarters and wild oats along with some seeds of other families for comparison. The period of heating was 15 minutes.

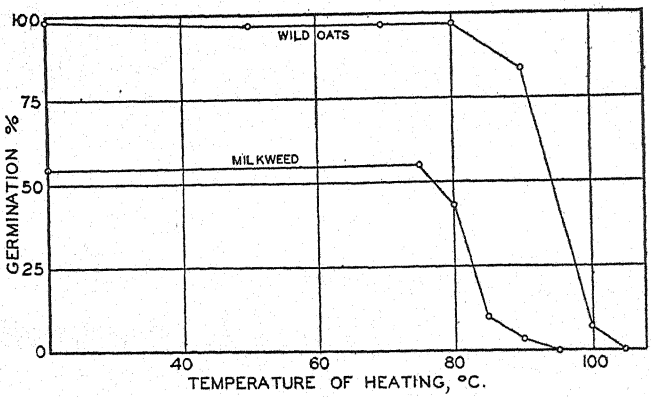

FIG. 3. *Effect of 15 minutes' heating on subsequent germination. (Graminae and Asclepiadaceae).*

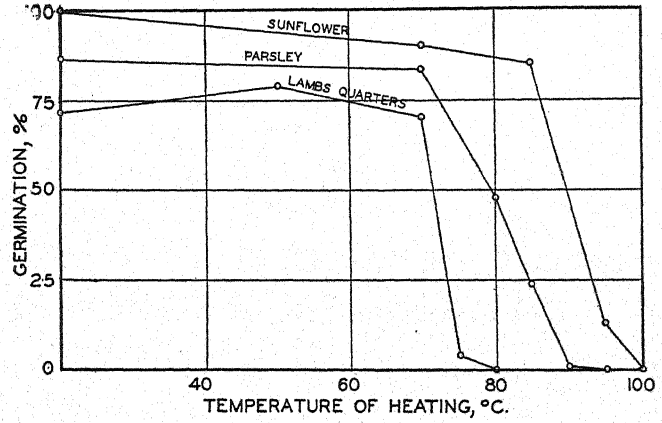

FIG. 4. *Effect of 15 minutes' heating on subsequent germination. (Compositæ, Umbelliferae and Chenopodiaceae).*

The effect of varying the time of heating was studied with seeds of Indian mustard, as shown in Table I. It is evident that increasing the time of heating has little effect on subsequent germination except at comparatively high temperatures.

TABLE I

GERMINATION OF INDIAN MUSTARD AFTER HEATING

Temp. of heating	Germination, %				
	70° C.	80° C.	85° C.	90° C.	95° C.
Period					
10 min.	96	92	89	87	—
20 min.	94	89	85	73	1
30 min.	97	94	89	56	—
60 min.	96	96	79	45	—

The approximate lethal temperatures under the conditions of the experiments are obtained by interpolation from Figs. 1–4 and are shown in Table II. These temperatures refer to seeds whose moisture content is such that they are in equilibrium with air at 25° C. and 50% relative humidity. The percentage moisture in the seed under these conditions will depend on the species, starchy seeds having a greater affinity for water than fatty seeds.

TABLE II

LETHAL TEMPERATURES

(15 minutes' heating)

Species	Initial germination, %	Lethal temperature, °C.
Indian mustard (*Brassica juncea*)	99	100
Wild oats (*Avena fatua* L.)	98	105
Wild mustard (*Brassica arvensis* (L)-Ktze)	91	95
Red root pigweed (*Amaranthus retroflexus* L.)	90	85
Lambs' quarters (*Chenopodium album* L.)	72	95
Stinkweed (*Thlaspi arvense* L.)	63	85
Milkweed (*Asclepias syriaca* L.)	54	95
Sunflower (*Helianthus annuus* L.)	100	100
Parsley (*Carum petroselinum* B & H.)	86	90–95

In addition to the species listed in Table II, lethal temperatures for fifteen minutes' heating were determined approximately for the following seeds: hare's ear mustard (*Conringia orientalis* (L) Dumort) 80° C.; ball mustard (*Neslia paniculata* (L) Desv.) 85° C.; Russian pigweed (*Axyris amaranthoides* L.) 80° C. The initial germination of these samples, however, was not as high as that of the other seeds described above.

The moisture content of wild mustard seed, when kept at 25° C. and under four different conditions of relative humidity, was determined. The results are compared with similar observations on wheat reported by Hottes and Wilson (1).

TABLE III

MOISTURE CONTENT OF SEEDS UNDER DIFFERENT CONDITIONS OF HUMIDITY

Relative humidity	Moisture content, %	
	Wild mustard, 25° C.	Wheat, 25° C.*
30%	5.38	—
50	6.57	9.8
60	7.18	14.4
70	7.78	ca.19

**Data from Hottes and Wilson (1).*

Discussion

The results show that in order to destroy the viability of a mixture of all the weed seeds tested, it would be necessary to use a temperature of slightly above 100° C. under the conditions of these experiments. Wild oats, the most resistant, showed 7% germination after 15 minutes' heating at 100° C.

but no germination after heating at 105° C. A temperature of 100° C. might be sufficient if the period of heating were increased to 20 minutes.

In some cases, a slight increase in *total* germination was noted after moderate heating (50° C.). Other investigators have observed an increase in the *rate* of germination subsequent to mild heat treatment.

It may be noted from the curves in Figs. 1–4 that the viability diminishes rapidly as the temperature of heating approaches the lethal point. This suggests that there is a critical temperature for each species below which a short period of heating has no appreciable effect on subsequent germination while higher temperatures cause rapid destruction of germinating power. The same effect is observed in earlier studies on corn, wheat, and tomato seeds, although Waggoner found a more gradual diminution in viability of radish seeds. These results are compared in Table IV with some of the results of the present work.

TABLE IV

EFFECT OF HIGH TEMPERATURES ON VIABILITY OF SEEDS

(Including data from earlier studies)

Temp. of heating, °C.		Germination, %										
		62.5	65	67.5	70	75	80	85	90	95	100	105
Corn	*(a)*	84	36	2	0							
Radish	*(b)*		91		83	74	64	56	28	0		
Tomato	*(c)*						95	38	3	0		
Wheat	*(d)*				96		100		8		0	
Red root pigweed	*(e)*				88	86	4	0				
Wild oats	*(e)*				95		96		84		7	0

(a) Moisture 12.5%. Heated 2 hours (5).
(b) Moisture 9%. Heated 30 minutes (6).
(c) Heated in air oven, 3 hours, (2).
(d) Moisture 9.9%. Heated 30 minutes (1).
(e) Present investigation. Heated 15 minutes.

The mechanism of the loss of viability on heating is unknown. Robbins and Petsch (5) studied the rate of coagulation of egg albumen at various temperatures in conjunction with their experiments on wheat and corn and observed some correlation between the rate of coagulation of albumen and the loss of germinating power in the seeds. It is inferred that heat coagulates the reserve protein of the seed and renders it incapable of being utilized.

The possibility exists that the loss of viability is more directly due to inactivation of the enzymes by heat. The resistance of enzyme preparations to heating at various temperatures is closely parallel to the behavior of seeds under the same conditions. According to Waksman (7), most enzymes are rapidly destroyed by heating in water at 70–80° C. When dried they will withstand temperatures of 100–120° C. or higher. Seeds treated under these conditions exhibit the same behavior with respect to viability.

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THE REACTION OF WHEAT HYBRIDS TO A SPRING FROST[1]

BY J. B. HARRINGTON[2]

Abstract

A moderate June frost proved highly efficient in the separation of wheat hybrid lines for resistance to spring frost. Eighty of 332 F_6 lines of H-44-24 × Reliance and fully 56 of 227 F_5 lines of (Reliance × Reward) × Reliance appeared to have the superior frost resistance of Reliance. Significant genetic differences in frost reaction were found among the lines of each of two new varieties.

Introduction

Cereal crop improvement has been going on for thousands of years and methods of breeding have advanced from primitive ways to the highly scientific technique of the present day. Until comparatively recent times the bringing together of widely different gene complexes through the hybridization of varieties and species found in widely separated regions of the earth was practiced infrequently. Now, however, such crosses form a distinct part of the modern breeding technique. This is particularly true in the case of the small grains. The old introduction method of improvement emphasized primarily the adaptation of a variety to a certain environment, and care was taken to obtain varieties from closely similar habitats. The new breeding technique, however, places major emphasis on character combinations and, in order to obtain a desired character, a variety quite lacking in general adaptation to the area under consideration may be used as a parent. Thus many of the hybrids possess various unsuitable attributes along with the desired character. The presence of these unfavorable attributes is not always evident or even suspected. Above all, these characters are not easy to avoid and sometimes, on account of linkage, are extremely difficult to eliminate.

An important example of an unfavorable character which accompanied a desirable one is susceptibility to damage from spring frosts. Resistance of spring wheat to spring frost has not been considered an important character, partly because its influence on the final outcome of a crop is obscure and partly because the well acclimated and introduced varieties used in the regions subject to late spring frosts are usually fairly resistant to cold.

Attention was drawn to the possible importance of spring frost resistance in cereals by Waldron (3) when he revealed the low frost resistance of Hope wheat as compared with the high resistance of some other varieties, and when he (Waldron 4) showed further that severe injury from spring frost had a

[1] *Manuscript received April 3, 1936.*
Contribution from the University of Saskatchewan, Saskatoon, Saskatchewan.
[2] *Professor of Field Husbandry.*

depressing effect on yield. Hope was produced by McFadden (2) of South Dakota by crossing Marquis with Yaroslav, an emmer wheat, for the purpose of combining the high resistance of emmer to black stem rust with the good qualities of Marquis. But frost susceptibility and some other unfavorable characters accompanied the rust resistance. In recent years Hope and a sister variety called H-44-24 have been used extensively in breeding programs in both Canada and the United States.

The importance of satisfactory tests of hybrid lines for resistance to frost is apparent. Comparative frost tests may be made in field nurseries or in controlled refrigeration chambers. Field tests are highly desirable but are difficult to bring about as it is largely a matter of chance whether a satisfactory low temperature condition occurs when the seedlings are in the very susceptible two- or three-leaf stage.

An exceptionally good opportunity to obtain critical data on the reaction of wheat hybrid lines to spring frost occurred on June 4, 1935, at Saskatoon, Saskatchewan. A fairly uniformly distributed frost of one to four degrees F. lasted for a period of four hours during the night. At this time a number of comparative plot tests of cereal varieties as well as several thousand rows of hybrid lines were in the two- and three-leaf stage. Notes were taken on the second and third days after the frost on many hundreds of plots and rows and the results proved extremely interesting.

The data from the various comparative plot tests of varieties were presented in a recent paper by the writer (1). The varieties ranged from highly frost resistant to highly frost susceptible ones in each crop. The comparative results on varieties of wheat showed a clear-cut relationship between ancestry and frost resistance.

In the present paper the summarized data from the hybrid lines will be presented. The data were obtained from two nurseries which were sown earlier than usual with the hope of obtaining a field test of comparative resistance to spring frost. The nurseries comprised several hundred F_5 and F_6 progeny plots of crosses involving both frost resistant and frost susceptible varieties of spring wheat. Check plots of the parental varieties were sown systematically throughout both nurseries.

Methods

The frost injury notes were taken on the hybrids three days after the frost occurred. A scale of 0 to 10 was used as follows: 0, all above ground parts of the seedling killed; 1, two leaves and culm severely injured; 2, two leaves severely and culm moderately injured; 3, two leaves severely and culm slightly injured; 4, two leaves severely injured; 5, one leaf severely and one moderately injured; 6, two leaves moderately, or one leaf severely and one slightly injured; 7, one leaf severely injured; 8, one leaf moderately injured; 9, one leaf slighlty injured; 10, no apparent injury. While the observations were on an individual plant basis a general average for all of the plants in a plot, that is for one line, was taken in each case.

Results

The frost injury data on 332 F_6 lines of the cross H-44-24 × Reliance are given in Table I. The mean of the lines was 8.0 ± .05 which was intermediate between the Reliance mean of 9.0 ± .10 and the H-44-24 mean of

TABLE I

DISTRIBUTION OF F_6 LINES OF THE CROSS H-44-24 × RELIANCE FOR INJURY DUE TO SPRING FROST AT SASKATOON IN 1935

Material	No. of plots	Distribution for degree of frost injury							Mean S.E.
		4	5	6	7	8	9	10	
F_6 lines	332		1	10	68	174	69	10	8.0 ± .05
Reliance	27					3	20	4	9.0 ± .10
H-44-24	27	1	1	3	10	11	1		7.2 ± .20

General mean = 8.01. $S.E._s$ = 0.91.

7.2 ± .20. Each of the three means differed from the others by highly significant odds. No correlation whatever was found between date of emergence and frost resistance index and nearly all of the plants were in the two-leaf stage and approximately of the same age at the time of the frost. The results indicate that there are significant genetic differences among the lines. It is reasonably safe to consider that the 79 lines occurring in Classes 9 and 10 possess the superior frost resistance of Reliance.

TABLE II

DISTRIBUTION OF F_5 LINES OF THE CROSS (RELIANCE × REWARD) × RELIANCE FOR INJURY DUE TO SPRING FROST AT SASKATOON IN 1935

Material	No. of plots	Distribution for degree of frost injury							Mean S.E.
		4	5	6	7	8	9	10	
F_5 lines	227	5	14	30	45	77	51	5	7.5 ± .09
Reliance	21			1	4	9	6	1	8.1 ± .20
Reward	79	2	7	24	37	9			6.6 ± .10

General mean = 7.32. $S.E._s$ = 1.35.

Table II shows frost injury data on 227 F_5 lines of the back-cross of Reliance × Reward on Reliance. The nursery where this material was grown was nearly 1000 feet from the one which furnished the data in Table I and the frost injury was more severe. The mean of the hybrid lines was 7.5 ± .09 while that of Reliance was 8.1 ± .20 and of Reward was 6.6 ± .10. Tests of significance show the three means to differ one from another by high odds. As with the Table I data, no correlation was found between date of emergence and degree of frost injury. The results indicate that genetic differences for frost reaction exist among the lines. Fully 56 of the lines appear to have the Reliance type of reaction and at least 49 show the Reward susceptibility to frost.

The two foregoing tables have demonstrated the separation of frost resistant and frost susceptible hybrid lines by means of a critical field test. Lacking a satisfactory frost resistance test, hybrid lines would be chosen without regard to frost resistance. In this way a new variety might lack uniformity as to frost resistance but this would not become apparent until a critical frost injury test was made. In 1935, at Saskatoon, 87 purified lines of three new stem-rust-resistant varieties of wheat were grown with some varieties used as checks in a randomized four replicate rod row plot test. The data obtained on resistance to the June 4 frost afforded an opportunity to note indications of genetic differences in frost resistance among the lines of two of the three varieties. The data are summarized in Table III.

TABLE III

SPRING FROST REACTION OF INDIVIDUAL PLANT LINES OF THREE NEW RUST RESISTANT VARIETIES OF SPRING WHEAT GROWN IN A RANDOMIZED FOUR REPLICATE ROD ROW PLOT TEST IN 1935

Material	No. of lines	Line distribution for frost injury*															
		4.2	4.5	4.8	5.1	5.4	5.7	6.0	6.3	6.6	6.9	7.2	7.5	7.8	8.1	8.4	Mean S.E.
Apex lines	25			2	1	2	2	2	9	3	3	1					6.2 ± .18
A 277 lines	14			3	4	2	2	2		1							5.4 ± .21
A 41 lines	48	4	7	24	6	3	2	1	1								4.9 ± .10
Apex										1							6.6 ± .49
A 277									1								6.3 ± .46
A 41						1											5.4 ± .40
Reliance																1	8.4 ± .62
Thatcher									1								6.3 ± .46
Marquis									1								6.3 ± .46
Reward				1													4.8 ± .35

* *Average of four replicates in each case.*

The standard error of a variety mean equals 7.4%.

χ^2 *tests:*

Apex lines vs. A 277 lines. $\chi^2 = 7.93$, $P = .02$.

A 277 lines vs. A 41 lines. $\chi^2 = 6.55$, $P = .01$.

The results reveal differences, which appear to be genetical, within the groups of lines. In Apex all of the lines rating 6.3 were less injured (odds of more than 21 to 1) than those rating 4.8. Sixteen of the 25 lines rated 6.3 or more. Lines rating 6.9 were less injured (odds of more than 45 to 1) than those in the 4.8 class, yet 4 lines rated from 6.9 to 7.2. These results show highly significant differences between lines with respect to frost reaction. In the A41 lines the distribution also deviates from the expected normal, though much less strikingly. In the A277 lines no significant deviation from normal expectation is shown.

It is worth noting also in Table III that there were significant differences between the line distributions of the three new varieties. All of these varieties originated from the cross (H-44-24 × Double Cross) × Marquis. Considering the parentage of H-44-24 and Double Cross, the three new hybrid varieties have inheritance ranging from the high frost resistance of Kanred, as represented by the Reliance reaction, to the relatively low resistance of H-44-24,

which is represented fairly well by the reaction of Reward (See 1). It is therefore to be expected that Apex, A277 and A41 differ from one another in frost reaction. These differences are only shown significantly in the reactions of the lines. Nevertheless the frost resistance order in which the three varieties occurred in their use as checks is the same as the order shown by the mean indices of the groups of lines.

Discussion

Owing to the present strong competition in the marketing of cereals, particularly wheat, it is necessary to have more efficient varieties as one means of reducing production costs. One mode of increasing the efficiency lies in adding disease resistance which is obtained usually from varieties introduced from distant parts of the world. Along with the disease resistance the introduced varieties bring undesirable characters. Frost susceptibility is one undesirable character contributed by the highly stem-rust-resistant emmer wheats.

As long as the wheats used on the western plains of North America were from hardy Russian stocks there was little need for paying attention to spring frost resistance as a varietal character. This situation changed when the hybridization method of breeding brought together the characters of distinctly unrelated wheats. The origination and use of Marquis wheat presumably introduced a measure of the frost susceptibility of the Indian parent, Hard Red Calcutta. Of late the origination of varieties resistant to stem rust by crossing common wheat with emmers has produced a further dose of spring frost susceptibility. Thus, while the increased disease resistance of the new varieties constitutes important progress, other characters possessed by these varieties may be definitely detrimental. It would now appear to be advisable to take steps to raise the general level of frost resistance. The high degree of frost resistance shown by Reliance and other varieties (1) derived from crosses with Kanred winter wheat indicates that a more extensive use of winter wheats in spring wheat breeding programs might be a very satisfactory procedure. In any event, it is obvious that adequate comparative tests for spring frost resistance are needed at the breeding institutions of the northern plains area of North America. It would seem that a plant breeding establishment should either be equipped with adequate refrigeration test chambers or else be able to have its new hybrid lines tested at some station which has such equipment.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

I. CROSSING RESULTS AND DESCRIPTION OF THE FIRST GENERATION HYBRIDS[1]

By J. M. Armstrong[2]

Abstract

Two species of tetraploid ($2n = 28$) and three varieties of hexaploid ($2n = 42$) wheat were crossed with *A. glaucum* ($2n = 42$), and *A. elongatum* ($2n = 70$), with an average crossing success of 18%. The seed obtained from tetraploid wheat × *A. glaucum* was slightly plumper and germinated better than that obtained from tetraploid wheat × *A. elongatum*. On the other hand, hexaploid wheat × *A. elongatum* gave decidedly plumper and better germinating seed than hexaploid × *A. glaucum*.

Grown under greenhouse conditions the F_1 hybrids proved to be self-sterile and perennial in habit, with hybrid vigor strongly marked. The hybrids were, in general, intermediate in morphological characters, but with somewhat more resemblance to *Agropyron* than to wheat. This dominance, whole or partial, was more noticeable in the *A. elongatum* than in the *A. glaucum* crosses. Dominance phenomena are discussed in relation to current theories.

Introduction

The practical possibilities of intergeneric crosses of the Hordeae have been emphasized by Dr. Meister, Director of the Central Station of Plant Breeding and Genetics at Saratov. The first successful crosses between *Triticum* and *Agropyron* were made in 1930 by N. V. Tzitzin. Since then the work in the U.S.S.R. has expanded rapidly and is now being carried on at several stations. The progress made has been reported by Tzitzin (5), Verushkine and Schekurdine (7) and Vakar (6). Their chief aim has been the creation of new forms of perennial wheat. For Canadian conditions the possibilities of obtaining new forms of forage crops by this method appear more attractive. Such new forms should be of considerable value in solving the soil-drifting problem of the prairie provinces. The present need is for a large-seeded forage crop which would be easy to establish and which would possess good soil-binding properties. Such a crop, besides restoring fibre to the soil, should furnish a good quality of feed grain in addition to fodder and pasture.

At the Central Experimental Farm during the summer of 1935, crosses were made between species of *Triticum* and *Agropyron*, and small populations of the various crosses were grown in the greenhouse during the past winter.

Description of Parents

The species of wheat and grass which were successfully crossed are shown in Table I. The wheat varieties were kindly supplied by the Cereal Division, Central Experimental Farm. They are pure line selections, highly homozygous for most morphological characters.

[1] *Manuscript received May 11, 1936.*
Contribution from the Division of Forage Plants, Central Experimental Farm, Ottawa. This contribution forms part of a co-operative investigation on the hybridization of Triticum and Agropyron undertaken by the Dominion Experimental Farm and the National Research Council, Canada. Presented at the Ottawa meeting of the Royal Society of Canada, May, 1936.

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TABLE I

SPECIES OF WHEAT AND *Agropyron* USED IN SUCCESSFUL CROSSES

Species	Variety	Polyploidy	Growth habit
Triticum dicoccum	Vernal Emmer	Tetraploid	Spring form
T. durum	Mindum	Tetraploid	Spring form
T. vulgare	Lutescens	Hexaploid	Spring form
T. vulgare	C.A.N. 1835	Hexaploid	Spring form
T. vulgare	Kharkov	Hexaploid	Winter form
Agropyron glaucum		Hexaploid	Perennial
A. elongatum		Decaploid	Perennial

C.A.N. 1835 requires a special explanation. It is a sixth generation segregate of the cross Pentad × Marquis, developed at the Winnipeg Rust Laboratory. Its grain is of high quality and it is quite rust-resistant.

While crosses were attempted between wheat and many species of *Agropyron*, only two species, *A. glaucum* and *A. elongatum*, were successful as male parents. These two species are indigenous to continental Europe and to Asia, but their forage possibilities have been tested at Ottawa for several years. They are tall-growing perennials of the bunch grass type with extensive, fibrous, root systems. Cross-pollination is usual although not obligatory. According to botanical descriptions these species are polymorphic, having well defined varieties and forms. Our limited observations have shown the existence of considerable variability. Both species have morphological features which render them highly drought-resistant. They are also claimed to be resistant to certain fungus diseases to which many species of wheat are susceptible. They have certain obvious faults however, such as coarse foliage and seed shattering.

A prospectus of the compatible species in the two genera indicates that, barring close genetic linkage, it should be possible to produce new varieties combining the desirable characters, perennial growth habit, drought and disease resistance, and vigorous root development of the grass parents with the characters, palatable foliage and large grain size of the wheat parents.

Crossing Results

The wheat species were used mainly as the female parents in the crosses, not only because the wheat varieties proved easier to emasculate and pollinate, but also because the *Agropyron* species furnished a more abundant supply of pollen. A few reciprocal crosses were attempted but with negative results.

It was observed that during the flowering period both *Agropyron* species shed their pollen on warm, bright afternoons. Large glassine bags were placed over a cluster of heads in the morning and in the afternoon a considerable quantity of pollen would be available for pollination. The pollen was placed in a petri dish or small envelope and applied to the stigmas of the emasculated wheat heads with a small camel's hair brush. For any given *Agropyron* species the flowering period lasted from seven to ten days.

To determine the error to be expected in crossing, a number of heads of each wheat variety were emasculated without subsequent pollination. Table II gives the results of this test. Out of a total of 2,645 flowers emasculated but not pollinated 19 flowers set seed, giving an error of 0.7%.

TABLE II

CROSSING ERROR AS DETERMINED BY EMASCULATION WITHOUT POLLINATION

Female parent	No. of flowers emasculated	No. of seeds set	Per cent error
Mindum	716	10	1.4
Vernal	196	2	1.0
C.A.N. 1835	637	0	0.0
Lutescens	942	7	0.7
Kharkov	154	0	0.0
Total	2645	19	0.7

The species of *Agropyron* which did not cross successfully with wheat are given in Table III. Nine such species were used in 34 combinations with an average of 193 flowers per combination. From the total of 9,597 flowers used in these crossing attempts, 36 seeds were obtained.

TABLE III

SPECIES OF *Agropyron* WHICH DID NOT CROSS SUCCESSFULLY WITH *Triticum*

Male parents	Female parents									
	Mindum		Vernal		Kharkov		Lutescens		C.A.N. 1835	
	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained	Flowers pollinated	Seeds obtained
A. desertorum	118	0	76	0	71	0	360	1	320	0
A. dasystachyum	239	0	—	—	—	—	120	1	575	1
A. caninum	78	1	—	—	—	—	142	1	183	1
A. imbricatum	40	0	64	4	—	—	40	0	60	1
A. repens	300	1	558	8	108	0	256	2	180	1
A. cristatum (Fairway)	280	0	286	6	156	0	460	2	420	0
A. cristatum (Commercial)	200	0	160	0	155	1	178	0	220	2
A. obtusiusculum	56	0	40	2	—	—	—	—	58	0
A. Richardsonii	40	0	—	—	—	—	—	—	—	—
Total	1351	2	1184	20	490	1	1556	7	2016	6
Per cent seeds obtained	—	0.15	—	1.69	—	0.20	—	0.45	—	0.30

Assuming these seeds to be of hybrid origin, the crossing success would be 0.54%, which is lower than the crossing error given in Table II. The possibility of selfing was further indicated by the plumpness of the seeds in question. In order to remove any doubt the seed was sown and the plants were grown

to maturity in the greenhouse. All proved to be wheat plants. The failu to obtain crosses between these species of *Agropyron* and wheat is clea due to incompatibility. The negative results obtained agree with those the U.S.S.R. investigators.

Table IV gives the results of the successful crosses of wheat with *A. glauc* and *A. elongatum*. Two strains of *A. elongatum* were used and the crossi results have been kept separate in the table. Considering the results as whole, a total of 9,648 flowers were pollinated and 1,784 seeds were obtain giving a crossing success of 18.5%. Deducting the crossing error of Tal II, the true success would be slightly under 18%.

TABLE IV

SUCCESSFUL CROSSES

Female parents	Male parents								
	A. glaucum			*A. elongatum* No. 820			*A. elongatum* No. 1083		
	Flowers pollinated	Seeds obtained	Per cent success	Flowers pollinated	Seeds obtained	Per cent success	Flowers pollinated	Seeds obtained	Per ce succe
Vernal	1196	414	34.6	1391	538	38.7	196	3	1.5
Mindum	1224	394	32.2	345	11	3.2	164	12	7.3
Kharkov	1041	122	11.7	239	25	10.5	328	45	13.7
Lutescens	1012	85	8.4	460	10	2.2	228	37	16.2
C.A.N. 1835	1099	70	6.5	268	16	6.0	457	2	.4
Total	5572	1085	19.5	2703	600	22.2	1373	99	7.2

In the crosses with *A. glaucum* a definitely higher degree of success wa obtained with the two tetraploid species in comparison with the three hexa ploid varieties. Of the latter, Kharkov, the winter form, crossed the mos successfully although the difference is not sufficient to indicate a greate compatibility.

In the crosses with *A. elongatum*, Vernal gave a very high and a very lo crossing success with the two *A. elongatum* strains, No. 820 and No. 108 respectively. Lutescens, on the contrary, crossed much more readily wit Strain No. 1083 than with Strain No. 820. The differences in both cases ar so marked that there is little doubt of their significance. In the case of th other wheat species, about the same degree of compatibility with the tw *A. elongatum* strains is apparent. The results would seem to indicate that th two strains of *A. elongatum* differ in their compatibility with certain varietie of wheat. Tzitzin (5) also reported that the different polymorphic forms o *A. glaucum* varied widely in their crossibility with various wheats.

Relative Plumpness of Wheat and Hybrid Seed

Table V gives the weight per ten kernels for the various crosses in com parison to the wheat parents. Clear-cut results in regard to seed plumpnes are apparent and can be briefly summarized. Tetraploid wheat gave slightly

plumper seed when crossed with *A. glaucum* than when crossed with *A. elongatum*. In the Vernal crosses the difference is not very strongly marked, but the large number of crossed seeds obtained enabled us to demonstrate a statistical difference. In the crosses with the hexaploid wheats the condition is reversed. The plumper seed was obtained from the *A. elongatum* crosses. While the number of crossed seed was not high enough to permit a statistical treatment yet the marked difference in kernel weight and the appearance of the seed (Plate I) show that this difference is highly significant.

TABLE V

MEAN WEIGHT OF HYBRID SEED IN COMPARISON TO SELFED SEED OF WHEAT PARENTS

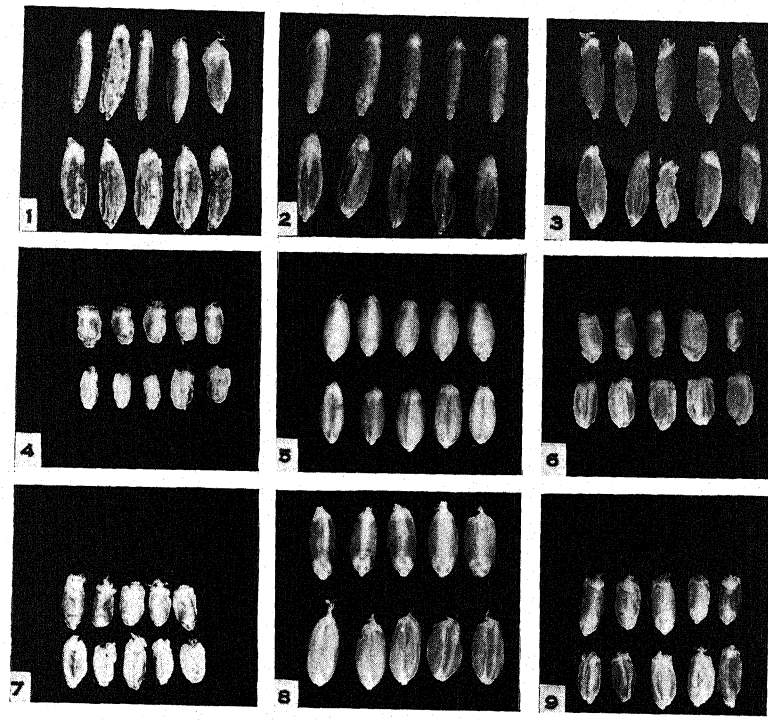
Material	Mean weight of 10 kernels, gm.	No. of kernels weighed
Vernal	.410	500
Vernal × *A. glaucum*	.194 ± .0054	250
Vernal × *A. elongatum*	.159 ± .0068	250
Mindum	.482	500
Mindum × *A. elongatum*	.167	23
Mindum × *A. glaucum*	.240 ± .0560	250
Kharkov	.304	500
Kharkov × *A. glaucum*	.057	122
Kharkov × *A. elongatum*	.109	70
Lutescens	.339	500
Lutescens × *A. glaucum*	.071	85
Lutescens × *A. elongatum*	.167	47
C.A.N. 1835	.280	500
C.A.N. 1835 × *A. glaucum*	.064	70
C.A.N. 1835 × *A. elongatum*	.103	18

In tetraploid wheat × *A. glaucum*, the hybrid seed is approximately one-half the weight of the seed of the respective wheat parents while in tetraploid wheat × *A. elongatum* it is about one-third. In hexaploid wheat × *A. glaucum* the hybrid seed is about one-fifth the kernel weight of the wheat parents while the same wheats crossed with *A. elongatum* gave hybrid seed about one-third to one-half the weight of seed of the wheat parents.

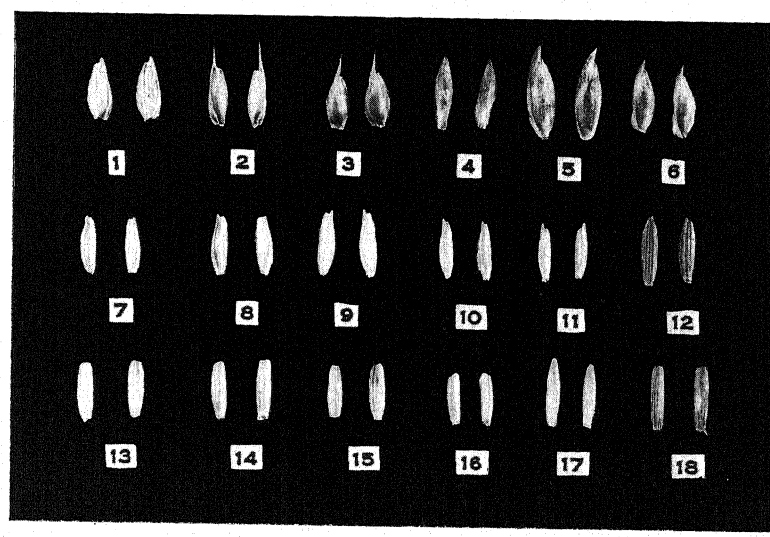
Germination of Hybrid and Parent Seed

Prior to the establishment of the F_1 populations in the greenhouse, germination tests were run on the parent and hybrid seed. The seeds were placed on sterilized quartz sand in petri dishes and the sand was kept moist with a 10% nutrient solution. The temperature was maintained at 65°–70° F. By this procedure it was hoped to promote development of the seedlings from the badly shrunken seeds as well as to make observations more convenient. Notes were taken daily on the rate of growth, rootlet number and percentage germination.

On the whole the germination was quite good, six of the crosses germinating more than 80%. By comparing Tables V and VI it will be seen that the plumper hybrid seed gave more satisfactory germination, this being most marked in the crosses involving *T. vulgare* varieties.



A



B

A. *Hybrid seed in comparison to seed of wheat parents.* 1. *Vernal* × *A. glaucum.* 2. *Vernal.* 3. *Vernal* × *A. elongatum.* 4. *Kharkov* × *A. glaucum.* 5. *Kharkov.* 6. *Kharkov* × *A. elongatum.* 7. *Lutescens* × *A. glaucum.* 8. *Lutescens.* 9. *Lutescens* × *A. elongatum.*

B. *Secondary glumes of wheat, Agropyron and hybrids from plants grown in the greenhouse with the exception of No. 5 which was field-grown.* 1. *Lutescens.* 2. *Kharkov.* 3. *C.A.N. 1835.* 4. *Vernal.* 5 *and* 6. *Mindum.* 7. *Lutescens* × *A. glaucum.* 8. *Kharkov* × *A. glaucum.* 9. *C.A.N. 1835* × *A. glaucum.* 10. *Vernal* × *A. glaucum.* 11. *Mindum* × *A. glaucum.* 12. *A. glaucum.* 13. *Lutescens* × *A. elongatum.* 14. *Kharkov* × *A. elongatum.* 15. *C.A.N. 1835* × *A. elongatum.* 16. *Vernal* × *A.*

TABLE VI

GERMINATION TESTS OF PARENTS AND F_1 HYBRID SEED

Material	No. of seeds sown	No. of seeds germinated	Germination, %	Times in days	Number of rootlets of germinating seeds						Greenhouse mortality
					5	4	3	2	1	0	
Vernal	75	75	100	4–5	72	3					0
Mindum	75	75	100	5–6	70	5					0
Kharkov	75	75	100	4–5	60	9	6				0
Lutescens	75	75	100	4–5	75						0
C.A.N. 1835	75	75	100	4–5	69	3	3				0
A. glaucum	96	86	90	8			55	23	8		0
A. elongatum No. 820	100	97	97	8			8	42	47		0
A. elongatum No. 1083	100	95	95	8			4	29	62		0
Vernal × *A. glaucum*	57	53	93	4–6		1*	52				0
Vernal × *A. elongatum*	59	51	87	4–6			41	9	1		0
Mindum × *A. glaucum*	57	55	96	4–5			53	1	1		1
Mindum × *A. elongatum*	19	9	47	5–7			9			1**	2
Kharkov × *A. glaucum*	63	40	64	6–9			5	16	19		1
Kharkov × *A. elongatum*	46	41	89	5–9			19	16	6		3
Lutescens × *A. glaucum*	50	31	62	5–8			3	4	24		5
Lutescens × *A. elongatum*	23	21	91	4–8			16	5			2
C.A.N. 1835 × *A. glaucum*	67	8	12	5–8					8		0
C.A.N. 1835 × *A. elongatum*	17	14	88	5–8			5	5	4		0

**This seedling had 2 plumules.* ***This seedling possessed a plumule.*

The cross C.A.N. 1835 × *A. glaucum* gave the very low germination of 12%. Upon dissecting the seeds that failed to germinate it was found that they were germless or completely lacking in embryos. This cross constitutes therefore an interesting case in the failure of double fertilization. Apparently at fertilization one male gamete fused with the polar nuclei to initiate endosperm development, but the other male gamete failed, in 88% of the cases, to effect fertilization of the egg.

A few other irregularities were observed in the germination tests. One seed from the cross Vernal × *A. glaucum* developed two plumules and four rootlets. This seedling subsequently developed into a normal F_1 plant. In another case a seed had a well developed plumule but no rootlets. A few seeds showed the reverse condition of one or more rootlets but no plumule. In other seeds the primordia ruptured the seed coat, but did not develop any further.

In number of seminal roots and in time required for germination, some significant differences were apparent. In wheat, all the seeds had three rootlets on the third day of the test, and by the fourth or fifth day two more rootlets had usually developed. A few seeds, particularly those of Kharkov, failed to develop the full complement of five rootlets. The grass species were slower to germinate than the wheat, and at the end of eight days had developed only one to three rootlets. The average rootlet number for *A. glaucum* was 2.5 and for the two strains of *A. elongatum* No. 820 and No. 1083 it was 1.6 and 1.4 respectively. In the time required for germination, the hybrid seed more closely approximated the wheat than the grass parents, although there

was less uniformity. In rootlet number they tended towards the grass parents since in no case were more than three rootlets per seed produced. It may be seen from Table VI that in the *T. vulgare* × *A. glaucum* crosses, which were characterized by very shrunken seeds, the majority of the seedlings had but one or two rootlets while in the *T. vulgare* × *A. elongatum* crosses, which gave plumper seed, most of the seedlings had two or three rootlets. If the degree of endosperm shrivelling had no effect on rootlet number the reverse condition would be expected, since *A. glaucum* has a higher rootlet number than *A. elongatum*. It may be concluded, therefore, that the factor or factors determining the rootlet number of the *Agropyron* parents is dominant over the allelomorphic factor in the wheat parents, but that the expression of this factor is modified by the endosperm condition of the hybrid seed.

Tzitzin reported that the hybrid seedlings which started with one rootlet invariably died before reaching maturity. Our results do not entirely agree with those of Tzitzin. The mortality noted in the last column of Table V was among the original one-rooted plants which died before there was any secondary root development, but, nevertheless the majority of one-rooted seedlings developed into normal plants.

Description of F_1 Plants

Growth and General Description

The F_1 seedlings, together with some parental material, were planted in a cool section of the greenhouse (50° F.) at the end of September. The hybrids tillered profusely, resembling the grass parents in this respect. Eight weeks after sowing, the hybrids and grass parents showed no indication of flowering stems, although all the wheats with the exception of Kharkov had done so. This winter habit was successfully broken by raising the temperature to 65°F. and exposing the plants to continuous light. Spike emergence of the hybrids commenced at the latter end of January and each plant flowered approximately two weeks after spike emergence.

Upon flowering, the lemma and palea separate widely and remain open ten days or longer. This type of behavior, characteristic of the open-pollinated *Agropyron* parents, may not be strictly inherited in the hybrids but may be due in part to sterility.

The female reproductive organs were normal in appearance, but the anthers, while appearing normal in size and color, failed to dehisce. Microscopic examination indicated about 5% good pollen. Complete self-fertility was found to be the rule in all the crosses.

One partially fertile hybrid occurred, however, in the C.A.N. 1835 × *A. elongatum* cross. In this plant the anthers dehisced normally and possessed 50–60% good pollen. Each head set about eight seeds which bore a general resemblance to those of the wheat parent.

All F_1 plants proved to be perennial like the *Agropyron* parents. The degrees of winter hardiness associated with this perennial growth habit will require tests this coming winter. Tzitzin found that the hybrids of *A. glaucum* with

spring wheat are much less frost-resistant than the corresponding hybrids with *A. elongatum* and that the hybrids with winter wheat were the hardiest of all. The dominance of the perennial habit in these crosses is of extreme importance not only because it permits the hybrids to be carried on indefinitely but also because, by means of cloning, a series of replicated experiments can be carried out to measure such characters as drought, frost and disease resistance.

Dominance Phenomena in the F_1 Hybrids

Eighteen pairs of characters were chosen for study. These were easily distinguishable in most of the parents. Some distinguish all the wheat varieties used from the *Agropyron* species, *e.g.*, habit (annual or perennial), spike compactness and number of rootlets in the seminal system; others, such as stem hollowness and width of glume, vary with one or both genera. The facts of dominance of the latter type of character were indeterminate in certain of the crosses.

Table VII shows the results of classifying the ten F_1 populations as to each of these 18 characters. The wheat condition is denoted by T, the *Agropyron* condition by A, intermediate by I, intermediate but more like wheat by IT and intermediate but more like *Agropyron* by IA.

Investigators in the field of *Triticum* × *Agropyron* hybridization have commented on the condition of dominance in the F_1 hybrids. Verushkine and Shechurdine (7) reported that "in all cases the F_1 plants showed the clear dominance of characters of the Couch grass and only a few plants occupied an intermediate position with regard to the character of their ear structure." Vakar (6) found that the dominance of the Couch grass characters was well defined in the hybrids. Tzitzin (5) on the other hand considered that in general the F_1 hybrids were intermediate with somewhat more resemblance to *Agropyron* than to wheat. An examination of the condition of dominance for the group of characters in Table VII confirms the latter author's observation. While it is true that there are more characters exemplifying dominance of the *Agropyron* than of the wheat parents, there is a large residue of intermediate characters.

The group of characters studied in which there is a clear dominance of the *Agropyron* parents will be dealt with first. Habit and the seminal root system have already been discussed and the dominance of the grass parents shown.

The time elapsing from spike emergence until anthesis differed markedly in the parents and hybrids. In wheat this period occupies four to six days and in the *Agropyron* 14–18 days. The hybrids closely resemble the *Agropyron* parents in this respect. Development during this period is not identical in wheat and *Agropyron*, as is clearly indicated by the different stages at which cytological preparations of the pollen mother cells must be taken. In both hybrids and the *Agropyron* parents three or four spikelets at the top of the spike must be showing clear of the sheath to be at the right stage for meiotic divisions, while the wheat species are at the right stage when the

TABLE VII

FREQUENCIES OF VARIOUS DEGREES OF DOMINANCE OF CHARACTERS IN TEN F_1 POPULATIONS OF *Triticum* × *Agropyron* CROSSES

F_1 population	Habit	Seminal root system	Spike density	Awning	Glume width	Beak	Keel	Barbs on keel	Glume adherence	Hollowness of culm	Leaf width	Number of leaf ridges	Prominence of ridges	Leaf Hairiness	Leaf scabrousness	Leaf posture	Waxiness	Time from spike emergence to anthesis	Frequencies of various degrees of dominance				
																			T	l T	I	I A	A
Lutescens × *A. glaucum*	A	A	I	–	IA	I	IT	T	A	A	IT	A	IA	IT	I	–	IA	A	1	3	3	2	6
Lutescens × *A. elongatum*	A	A	IA	–	I	A	IA	I	A	A	I	I	IA	T	I	A	–	A	1	0	5	3	7
C.A.N. 1835 × *A. glaucum*	A	A	I	I	I	I	IT	IT	A	IA	I	IA	IA	T	I	–	A	A	1	2	6	3	5
C.A.N. 1835 × *A. elongatum*	A	A	IA	A	I	A	IA	I	A	A	A	IA	A	I	I	A	–	A	0	0	4	3	10
Kharkov × *A. glaucum*	A	A	I	I	I	I	IT	IT	A	I	IT	IA	I	I	I	–	A	A	0	3	8	1	5
Kharkov × *A. elongatum*	A	A	IA	A	I	A	IA	I	A	A	IA	IA	A	I	I	A	–	A	0	0	4	4	9
Mindum × *A. Glaucum*	A	A	I	I	I	I	IT	IT	A	I	IT	I	I	I	I	–	A	A	0	3	9	0	5
Mindum × *A. elongatum*	A	A	IA	IA	I	A	IA	IT	A	–	A	I	A	A	IA	A	–	A	0	1	2	4	9
Vernal × *A. glaucum*	A	A	I	I	–	I	IT	I	–	–	A	I	I	I	IA	–	–	A	0	1	7	1	4
Vernal × *A. elongatum*	A	A	IA	A	A	A	IA	I	–	–	A	IA	A	I	A	A	IA	A	0	0	2	4	10

spike shows only a barely perceptible swelling within the sheath. Consequently when the wheat spike emerges from the sheath the gametophytic phase is fairly far along, while the *Agropyron* species pass through the whole gametophytic stage after emergence from the sheath. The close agreement of the *Agropyron* parents and hybrids with respect to this part of the developmental cycle is fairly definitive of the whole cycle.

Glume adherence is another character for which the *Agropyron* parents showed complete dominance. The character depends mainly on the degree of development of the collar at the base of the glume. In the two *Agropyron* species and *T. dicoccum*, the outer glumes adhere closely to the flowering glumes while in the remaining wheat species the glumes adhere loosely. The expression of this character determines the threshing qualities of the species.

Glaucousness or waxy bloom, a character which imparts a dull, bluish-gray color to the plants previous to the ripening period, is characteristic of *A. glaucum* and is also present in *T. dicoccum*. In the four crosses in which this character is brought in by the *Agropyron* parent it is wholly or partially dominant, but in the cross in which it is brought in by the wheat parent, absence of waxiness is partially dominant.

The leaf blade attitude is very characteristic in *A. elongatum*, being of the "flag leaf" type. During the early growth stages the leaf blades are semi-vertical in attitude while on the culms they are retrorse. This character probably depends upon the interaction of several factors. In the five wheat × *A. elongatum* crosses the leaf attitude of the *Agropyron* parent is completely dominant.

Most of the characters for which intermediacy is exhibited in the F_1 hybrids are quantitative, *e.g.*, spike density, glume width, leaf width and leaf scabrousness. Partial dominance of the *Agropyron* parents is frequent, however, for even this type of character.

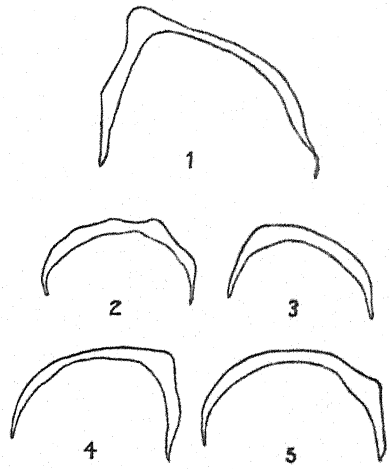
The most striking feature in this study is the contrasting degrees of dominance exhibited by the two sets of crosses, *Triticum* × *A. glaucum* and *Triticum* × *A. elongatum* for certain characters. This may be exemplified by the two characters, awning and the keel of the secondary glume.

All the wheat varieties except Lutescens are awned types, while the two *Agropyron* species are awnless. In the crosses of *A. glaucum* parentage all the hybrids are strongly tip-awned; that is, the intermediate condition prevails. In the crosses of *A. elongatum* parentage, on the other hand, the hybrids are completely awnless with the exception of *T. durum* × *A. elongatum* in which short tip awns are present on the lemmas of the upper spikelets.

Figs. 1–5 illustrate the keel condition in the parents and hybrids of the Karkov crosses. The cross sections were taken equidistant from the tip and base of the secondary glumes. In Kharkov the keel is strongly developed with the face and inner side of the glume approximately at right angles. In the *Agropyron* species the keel is weakly developed and the face and inner

side tend to be rounded without subtending a definite angle. The F_1 hybrids of Kharkov × *A. glaucum* tend to resemble the wheat parent both in the keel development and the angle of the face and side while in the F_1 of Kharkov × *A. elongatum* the keel is distinctly more like *A. elongatum*.

Other characters for which *A. elongatum* seems to exert a stronger influence than does *A. glaucum* are spike density, beak type and leaf width. The total effect is that the *A. elongatum* hybrids are distinctly more *Agropyron*-like than the *A. glaucum hybrids*.



FIGS. 1–5. *Cross-sections of secondary glumes drawn with the aid of a camera lucida.* 1. *Kharkov.* 2. *A. glaucum.* 3. *A. elongatum.* 4. *Kharkov* × *A. glaucum.* 5. *Kharkov* × *A. elongatum.*

Several theories devised to explain the phenomena of dominance have been advanced in recent years by various geneticists. Fisher (1) has advanced the theory of the evolution of dominance. His theory rests on the known frequent occurrence of mutations which are recessive to the wild type and deleterious to the organism. He believes that natural selection, acting over long periods of time, has operated to select appropriate modifying factors to make the heterozygotes equally viable to the homozygous wild type and indistinguishable from it.

Fisher's theory of the modification of a character to produce dominance implies that the heterozygotes were originally less viable than the wild type. This lack of viability is not found in the type of character dealt with in Table VII. Many species and varieties of wheat and *Agropyron* are known which possess the extreme alternative characters in awning, glume shape, beak length and spike density without any differential vigor being associated with them. Hence the dominance phenomena in the wheat × *Agropyron* hybrids cannot be adequately explained on Fisher's theory.

Wright (8) considers that it is unnecessary to formulate a theory for the evolution of dominance, but that final character expression depends upon the degree of completion of a chain of physiological processes under genic control. He postulates that the genes act through the control of specific catalysts, enzymes, and that the rate of any physiological process in the chain depends upon the proportion of catalyst to substrate. If the substrate is low in amount and the catalyst in excess, the further increase of the amount of the catalyst should exhibit extreme dominance. Conversely, if the substrate is in excess and the catalyst is low in amount the catalyst will be kept continually in combination and the gene will exhibit intermediacy.

This theory of the control of rate by limiting factors seems to be especially applicable in explaining the different degrees of dominance exhibited for

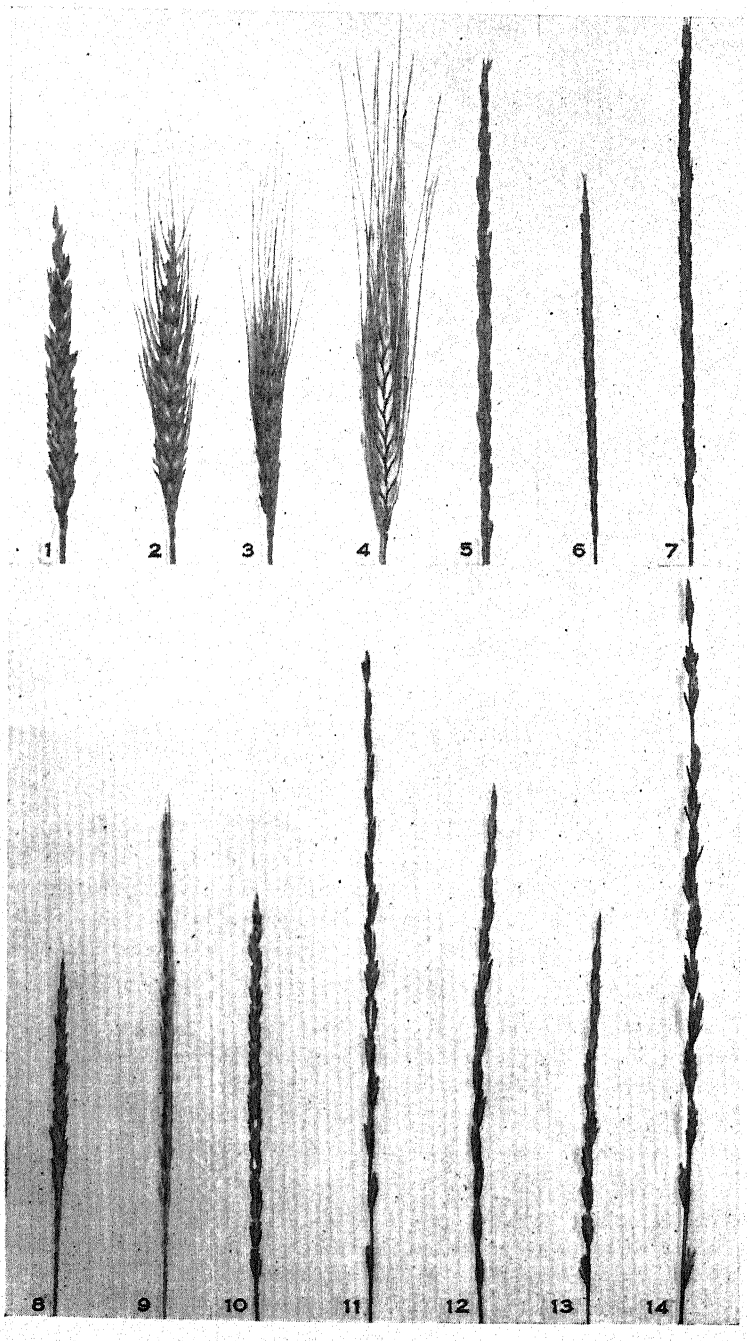


FIG. 6. *Heads of wheat and Agropyron parents and F_1 hybrids.* 1. *Lutescens.* 2. *Kharkov.* 3. *C.A.N. 1835.* 4. *Vernal.* 5. *A. glaucum.* 6. *Lutescens* × *A. glaucum.* 7. *Kharkov* × *A. glaucum.* 8. *C.A.N. 1835* × *A. glaucum.* 9. *Vernal* × *A. glaucum.* 10. *Lutescens* × *A. elongatum.* 11. *Kharkov* × *A. elongatum.* 12. *C.A.N. 1835* × *A. elongatum.* 13. *Vernal* × *A. elongatum.* 14. *A. elongatum.*

certain characters in the wheat × *A. glaucum* and wheat × *A. elongatum* crosses. *A. glaucum* is a hexaploid species ($2n = 42$) and *A. elongatum*, a decaploid ($2n = 70$), and on *a priori* grounds it might be presumed that there is a greater replication of genes affecting the same character in the latter species than in the former. Peto (3) has presented cytological evidence to show that *A. glaucum* is characterized by fairly regular bivalent formation at the first reduction division in the pollen mother cells while *A. elongatum* is characterized by frequent quadrivalent associations and configurations involving as many as eight chromosomes. These multiple chromosome configurations in *A. elongatum* indicate extensive chromosome replication with corresponding genic replication.

On the above cytological grounds we may conclude that in the *A. elongatum* crosses there is a numerical preponderance of genes causing awnlessness, and the catalyst controlled by them is more apt to be in excess, with a consequent dominance of awnlessness. In the *A. glaucum* crosses the catalyst would be lower in amount owing to the reduced number of genes causing awnlessness, with consequent intermediacy for the character. Wright's theory applied to our results assumes the accumulative effect of genes in their catalytic activity. There is ample proof for the validity of such an assumption in the work of Stern (4) and Muller, League and Offermann (2) with *Drosophila melanogaster* in which an accumulation of recessive genes brought about an approach to the wild type.

Acknowledgments

The writer wishes to acknowledge his indebtedness to Dr. L. E. Kirk, Dominion Agrostologist, for kindly supervision throughout the course of these studies; to Dr. F. H. Peto for close co-operation and assistance, and to Dr. L. P. V. Johnson for reading the manuscript.

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HYBRIDIZATION OF *TRITICUM* AND *AGROPYRON*

II. CYTOLOGY OF THE MALE PARENTS AND F_1 GENERATION[1]

By F. H. Peto[2]

Abstract

Meiosis was studied in *A. glaucum* ($2n = 42$), *A. elongatum* ($2n = 70$), and in the F_1 of these species crossed on varieties of *T. dicoccum*, *T. durum* and *T. vulgare*. In *A. glaucum* a large proportion of the chromosomes formed bivalents with occasional univalents and quadrivalents. *A. elongatum* was very unusual in that uni-, bi-, tri-, quadri-, quinqui-, sexa-, and octavalent configurations were observed. With one exception the *A. glaucum* × *Triticum* hybrids averaged 4.8–6.2 bivalents per nucleus, thus indicating partial homology between one set of chromosomes from each of the parents. In the *A. elongatum* × *Triticum* hybrids, numerous multivalent configurations were observed and it was concluded that auto- as well as allosyndesis had occurred. Approximately one set of chromosomes remained unpaired in one collection of *T. dicoccum* var. Vernal × *A. elongatum* and approximately two sets remained unpaired in crosses between three varieties of *T. vulgare* and *A. elongatum*.

Two of the crosses exhibited an abnormally small amount of pairing, an effect most plausibly attributed to the reaction of genetic factors limiting prophase pairing.

Tentative conclusions have been made regarding the origin and genetic constitution of *A. elongatum* from the pairing behavior of the chromosomes in this species and its hybrids. It appears likely that *A. elongatum* arose through hybridization between hexaploid and tetraploid species of *Agropyron* with subsequent chromosome doubling. An alternative explanation is also suggested.

Introduction

Studies on the conjugation of the parental chromosomes in hybrids have been particularly valuable in determining the phylogenetic relations between the various species of *Triticum* as well as the relations of these to species of *Aegilops*, *Secale* and *Haynaldia*. During the summer of 1935, successful crosses were made between certain species of *Triticum* and *Agropyron* (2). Since the material obtained afforded an opportunity of advancing our knowledge of the genetic relation of *Agropyron* to *Triticum*, analyses of chromosome conjugation in these hybrids were made during the past winter.

The conclusions arrived at by this method are based on the assumption that the extent of pairing of the chromosomes at zygotene is conditioned by the degree of genetic homology of the chromosomes involved. Hence, if normal pairing is observed at metaphase of the heterotypic division, it is assumed that there is at least partial genetic homology between the paired chromosomes. Numerous cases have, however, been reported where certain genes may limit or prevent prophase pairing. In consequence, a reduced amount of pairing or a lower chiasma frequency may not always indicate a proportional decrease in genetic homology. This danger of misinterpretation

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. This contribution forms part of a co-operative investigation on the hybridization of Triticum and Agropyron undertaken by the Dominion Experimental Farms and the National Research Council of Canada. Presented at the meeting of the Royal Society of Canada, Ottawa, May, 1936.

[2] *Biologist, National Research Laboratories, Ottawa.*

may be minimized by studying an adequate number of similar crosses, since it is unlikely that they would all possess the genic complex necessary to suppress normal pairing.

Literature Review

It was reported by Tzitzin (7) that *A. glaucum*, *A. trichophorum*, *A. elongatum* and *A. junceum* crossed readily with various *Triticum* species while no successful crosses were made with any of the other species of *Agropyron*. From a consideration of these facts and the morphological similarities of the above four species to *Triticum* he concluded that these *Agropyron* species should be included in the genus *Triticum*.

Vakar, Krot and Brekina (8) studied the meiotic behavior of the F_1 of *T. durum* × *A. elongatum*, *T. vulgare* × *A. elongatum*, and *T. vulgare* × *A. glaucum*. They found an average of 10 bivalents at the heterotypic metaphase and concluded that conjugation occurred between the sets A and B of *Triticum* with similar sets in *Agropyron*. Sapehin (6) conducted studies on similar hybrids but evidently used a different form of *A. elongatum* since the F_1 of a cross with *T. vulgare* contained 49 instead of 56 chromosomes. Twenty-one bivalents were observed at heterotypic metaphase in this cross. In the cross *T. vulgare* × *A. glaucum* he obtained very different results to Vakar *et al.* since 2–3 bivalents instead of 10 were observed.

Material and Methods

The crosses were made in the Division of Forage Plants at the Central Experimental Farm, Ottawa, during the summer of 1935 and have been reported by Armstrong in the first paper of this series (2). The male parents, *A. glaucum* and *A. elongatum* and nine different crosses with species of *T. dicoccum*, *T. durum* and *T. vulgare* were grown in the greenhouses of the Division of Forage Plants and the National Research Laboratories during the past winter.

Anthers at the proper stage were collected and fixed in absolute alcohol 3 parts and glacial acetic acid 1 part. They were transferred to 75% alcohol after 12–24 hours and stained and made permanent by McClintock's method (4).

Ten, and in some cases twenty, nuclei of each parent and hybrid were analyzed by examination of side views at heterotypic metaphase. Interpretation of polar views was never used since it was found to be impossible to detect and interpret multiple configurations in this manner.

Photomicrographs were used almost entirely for purposes of illustration. It may be impossible in many photomicrographs for the reader to analyze these nuclei completely, nevertheless they should indicate the validity of the author's analysis more accurately than do camera lucida drawings. Two camera lucida drawings are, however, included to supplement the photomicrographs. A Zeiss apo. 1.3 mm. objective and 8 × ocular was used for magnifications below 1000 × while a 25 × ocular was used for photomicro-

graphs of a higher magnification. A green (V.G.3) filter and orange (O.G. filter made by Jenaer Glaswerk were used in front of the light source. Tl pictures were taken on Eastman Panatomic film and developed in D72.

Observations

A. glaucum ($2n = 42$)

Ten metaphase nuclei were completely analyzed and the average frequencie for the various configurations are given in Table I. Four of the nuclei con tained 21 bivalents, while of the remainder, five contained two univalents and one contained four univalents. A typical nucleus is shown in Fig. 1 which contains 19 ring-shaped bivalents with two chiasmata each, one rod shaped bivalent with one chiasma and two univalents. The chiasma frequency per bivalent in this nucleus is 1.9 which is slightly higher than the average o 1.7. Two hundred tetrads were examined and 4.5% contained micronuclei It is apparent that only a small proportion of the univalents present behaved abnormally enough to be excluded from the tetrad nuclei.

Two of the metaphase nuclei contained each a chain of four chromosomes, and in other nuclei which were not completely analyzed rings of four chromosomes were seen. This suggests that at some time an interchange of segments between non-homologous chromosomes had occurred.

A. elongatum ($2n = 70$)

Chromosome conjugation in this species is very complex, since multiple configurations are always present. The chromosome number was accurately checked by counts at heterotypic anaphase. Seventy chromosomes can be clearly seen in Fig. 3. Complete nuclei could be analyzed wherever it was possible to find cells in which the contents had been spread out by pressure when the cell was in an oblique position relative to the equatorial plate. Two such nuclei are shown in Figs. 2 and 4. Their constitution is given in the legend. The average frequencies of the various associations for the 10 nuclei analyzed are as follows: 2.0 univalents, 22.1 bivalents, 0.6 trivalents, 3.0 quadrivalents, 0.2 quinquivalents, 0.7 sexavalents and 0.6 octavalents. It is important to note the preponderance of associations with an even valency, the number of quadrivalents being particularly high. Rings of eight chromosomes are of fairly frequent occurrence and in one instance two such rings were observed in a single nucleus. This is believed to be the first time octavalent associations have been observed in a naturally occurring species of Gramineae. A ring of eight at metaphase is shown in Fig. 5 and the same configuration is seen in Fig. 4 at a much lower magnification. An octavalent at diakinesis is shown in Fig. 6 and a diagrammatic interpretation is also included. Further terminalization of such a configuration may form either a ring of eight or a ring of six with an appended pair. The significance of these configurations will be discussed later in relation to the pairing condition found in the hybrids with *Triticum*.

The homotypic division appeared to be relatively normal. Two hundred tetrads were examined and 9% of these had micronuclei. This is twice the percentage found in *A. glaucum*, a result not unexpected considering the complex pairing observed in *A. elongatum*.

Tetraploid Triticum × *A. glaucum* ($2n = 35$)

In the F_1 of *T. dicoccum* var. Vernal × *A. glaucum* there was an average of 20.4 univalents, 6.2 bivalents and 0.8 trivalents per nucleus (Table I) while in the F_1 of *T. durum* var. Mindum × *A. glaucum*, 22.0 univalents, 5.5

TABLE I

CHROMOSOME ASSOCIATIONS IN PARENTAL SPECIES AND F_1 OF *Triticum* × *Agropyron* HYBRIDS

Parent or cross	Chr. No. ($2n$)	No. of cells	Associations of								Configurations	Chrs. associated
			1	2	3	4	5	6	7	8		
A. glaucum	42	10	1.4	19.9		0.2						
A. elongatum	70	10	2.0	22.1	0.6	3.0	0.2	0.7		0.6		
T. dicoccum var. Vernal × *A. glaucum*	35	20	20.4	6.2	0.8						7.0	14.8
T. durum var. Mindum × *A. glaucum*	35	20	22.0	5.5	0.5	0.05					6.1	13.0
T. vulgare var. Lutescens × *A. glaucum*	42	20	30.5	4.8	0.6						5.4	11.4
T. vulgare var. Kharkov × *A. glaucum*	42	20	30.3	5.6	0.01						5.6	11.2
T. vulgare var. C.A.N. 1835 × *A. glaucum*	42	20	39.3	1.2	0.05						1.3	2.6
T. dicoccum var. Vernal × *A. elongatum*												
Collection No. 1	49	7	6.6	12.1	3.6	1.1	0.6				17.4	42.4
Collection No. 2	49	7	17.7	8.3	3.0	0.7	0.6				12.6	31.3
T. vulgare var. Kharkov × *A. elongatum*	56	10	19.5	9.5	3.0	1.6	0.5				14.6	36.9
T. vulgare var. C.A.N. 1835 × *A. elongatum*	56	10	13.0	11.7	3.4	1.7	0.4	0.1			17.3	43.0
T. vulgare var. Lutescens × *A. elongatum*	56	10	14.2	10.6	3.4	1.1	0.8	0.2		0.1	16.2	41.8

bivalents, 0.5 trivalents and 0.05 quadrivalents were present. The presence of approximately 5–6 bivalents indicates partial homology between Set A or B of *Triticum* and one of the sets derived from *A. glaucum*. The occurrence of trivalents would be expected since quadrivalents were occasionally observed in *A. glaucum*. Partial homology would exist, therefore, between certain pairs of chromosomes constituting the gametes of *A. glaucum* and these would form the observed trivalents on pairing with additional homologous chromosomes contributed by the *Triticum* parent.

The chiasma frequency per bivalent for Mindum × *A. glaucum* was only 1.2, while *A. glaucum* had a chiasma frequency of 1.7. The closeness of pairing in the hybrid, therefore, was much weaker than that found in the male parent. In addition there was almost complete termination of the chiasmata in the hybrid since the coefficient was 0.96.

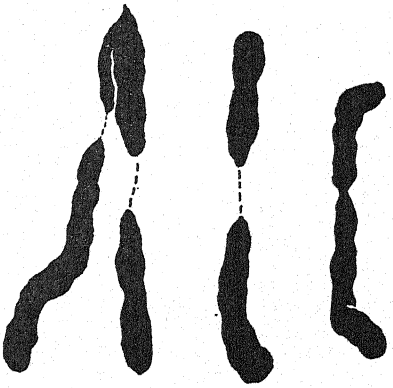
The appearance of the bivalents and trivalents in the Mindum cross can be seen in Fig. 8. This cell was scarcely typical since three trivalents and only two bivalents were present, whereas in other cells the occurrence of trivalents was relatively infrequent as may be seen in Table I. A high degree of pollen sterility would be expected in this cross since more than half of the pollen cells contained micronuclei when examined just prior to the first nuclear division.

Hexaploid Triticum × *A. glaucum* ($2n = 42$)

The meiotic behavior of the F_1 of the varieties Lutescens and Kharkov *(T. vulgare)* crossed with *A. glaucum* was very similar. In the Lutescens cross an average of 30.5 univalents, 4.8 bivalents and 0.6 trivalents were present, while in the Kharkov cross, 30.3 univalents, 5.6 bivalents and 0.01 trivalents were present. Although there were fewer bivalents in the former, this was compensated for by the increased number of trivalents, so that the average number of chromosomes associated in one was 11.4 and the other 11.2. The data in these crosses corroborated, therefore, the conclusion arrived at for tetraploid *Triticum* × *Agropyron* which indicated partial homology between Set A or B of *Triticum* and one of the sets derived from *A. glaucum*. In the hexaploid crosses the presence of a C set of chromosomes accounted for the additional univalents observed.

The chiasma frequency per bivalent in Lutescens × *A. glaucum* was 1.3 and the coefficient of terminalization 0.78, while in Kharkov × *A. glaucum* the chiasma frequency was 1.0 and the coefficient of terminalization 0.94. These differences are of doubtful significance.

Pairing of chromosomes of unequal length was observed in six of the 20 nuclei analyzed in the Lutescens cross. Two heteromorphic bivalents and one heteromorphic trivalent are shown in Text–Fig. 1. These observations indicate that structural changes of considerable magnitude have taken place if these chromosomes have evolved from a common primary set.



TEXT-FIG. 1. *Lutescens* × *A. glaucum*, *one heteromorphic trivalent and two heteromorphic bivalents*, × 2560.

The frequency of pairing in the cross C.A.N. 1835 × *A. glaucum* was strikingly different from the above two crosses since an average of only 1.2 bivalents per nucleus was observed. No pairing whatever was found in eight of the twenty nuclei examined. A typical asynaptic nucleus is shown in Fig. 9 in which 42 univalents can be counted at heterotypic metaphase. The largest number of bivalents observed was four.

An average of 1.3 chiasmata per bivalent was observed and these had all completely terminalized. The significance of this abnormally low degree of pairing in this cross will be discussed later.

Tetraploid Triticum × *A. elongatum* ($2n = 49$)

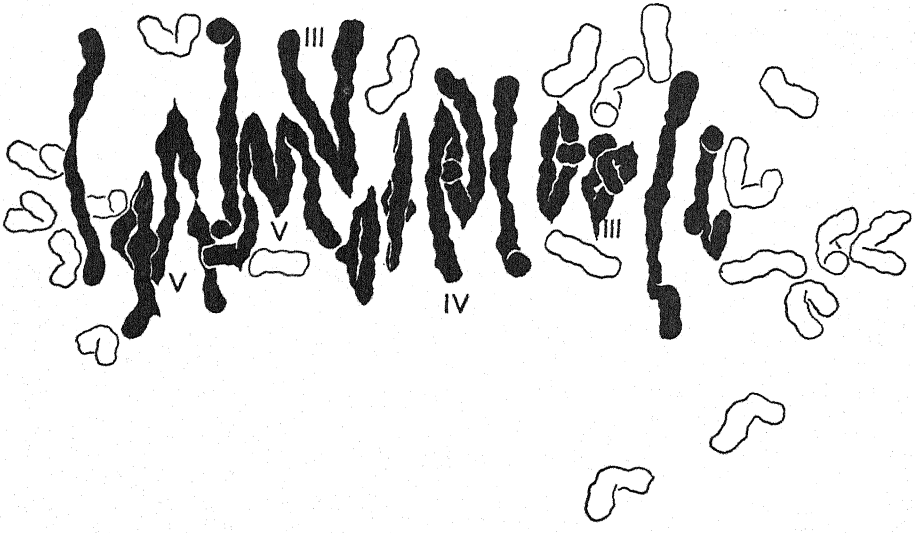
T. dicoccum var. Vernal and *T. durum* var. Mindum were successfully crossed on *A. elongatum*, but only the Vernal × *A. elongatum* cross has been examined cytologically. This cross is particularly interesting in that different collections gave significantly different results. In Collection 1 there were only 6.6 univalents while in Collection 2 there were 17.7 univalents. In the latter there was considerable reduction in the number of bivalents, but the numbers of trivalents, quadrivalents and quinquivalents were very similar in both collections. The identity of individual parent plants was not recorded in these initial crosses, and it is possible that intravarietal differences may exist in the parents. The manner in which such differences could affect the pairing of the chromosomes will be considered in the discussion.

The heterotypic division is not illustrated or described here, since it did not differ from that of other *A. elongatum* crosses, which are described later, except in the frequency of the various configurations. This cross is, however, used to illustrate the behavior in the homotypic division. Fig. 12 shows a typical homotypic metaphase plate from Collection 1. The majority of the chromosomes are undergoing the normal division at the equatorial plate. Single unsplit chromosomes may be seen scattered throughout the cytoplasm and these undoubtedly arose from the univalents which had already split in the heterotypic division. The splitting of univalents in the heterotypic division is shown in Figs. 13 and 15. A homotypic anaphase plate, in which the univalents are no longer distinguishable, is shown in Fig. 11. These univalents nevertheless appear to be responsible for the numerous micronuclei which can be observed in the tetrad stage. A typical tetrad is shown in Fig. 10. The presence of these micronuclei is usually associated with pollen sterility in wide crosses.

Hexaploid Triticum × *A. elongatum* ($2n = 56$)

The F_1 plants of *A. elongatum* crossed with Kharkov, C.A.N. 1835 and Lutescens were available for study. These hybrids had 56 chromosomes, and considerable difficulty was experienced in obtaining metaphase plates in which the chromosomes were separated sufficiently to permit accurate interpretations of the various associations. Ten nuclei of each cross were found in which the whole complement of chromosomes could be resolved. Fig. 14 shows a metaphase plate of C.A.N. 1835 × *A. elongatum*, while Figs. 16 and 17 show plates of Lutescens × *A. elongatum*. The results of the analysis of these plates are given in the legend and the valencies of certain configurations are indicated by numerals on the photomicrographs. While the reader will not be able to interpret all the configurations present in these photomicrographs, he will recognize the possibility of complete analysis of the original preparations by careful study of the configurations at different foci. A

camera-lucida drawing of a nucleus of Kharkov × *A. elongatum* is shown in Text-Fig. 2 in which the configurations have been drawn separately to show clearly the interpretation of configurations of various valencies. The univalents are unblocked, the bivalents are blocked and the multivalent configurations are identified by Roman numerals.



TEXT-FIG. 2. *Kharkov × A. elongatum, 20 × I, 8 × II, 2 × III, 1 × IV, 2 × V, × 1490.*

The univalents split equationally in the heterotypic division and two slightly different anaphase stages are shown in Figs. 13 and 15. In the former, several of the chromosome halves have already become widely separated and it is believed that separation is usually complete prior to cytokinesis. In Fig. 15 the split is just apparent in the lagging univalents. The univalents which have divided equationally in the heterotypic division do not become aligned properly at the equatorial plate at the homotypic metaphase but appear to be scattered throughout the cytoplasm as illustrated for the F_1 of Vernal × *A. elongatum*. Numerous micronuclei are found in the tetrads and young pollen nuclei and consequently a large proportion of sterile pollen might be expected.

It is important to note how closely the frequencies for the various configurations check in these three crosses. The wheat varieties used have widely different origins and their genetical differences might have been expected to be reflected in the relative frequencies of particular configurations. The fact that their behavior is so similar indicates that most of the varietal differences were the result of individual gene changes rather than chromosome aberrations. It should also be noted that these hybrids possess very few quadrivalents (1.1–1.6) but many more trivalents (3–3.4), whereas the situation was reversed in the *A. elongatum* parent where the trivalents were scarce but an average of three quadrivalents per nucleus was observed.

It would not be valid to conclude from the large number of chromosomes associated in these *A. elongatum* crosses, that there was a higher degree of homology between the parental chromosomes involved in these crosses than in the *A. glaucum* crosses where there were relatively few associations. Autosyndesis of the *A. elongatum* chromosomes is undoubtedly responsible for a considerable proportion of the associations observed. The relative proportions of allo- and autosyndesis cannot be determined solely by cytological studies of the hybrids themselves. However, if the genetical constitution and origin of *A. elongatum* can be determined and the evidence from a large number of interspecific and intergeneric crosses in the tribe Hordeae correlated, then it may be possible to estimate the relative proportions of auto- and allosyndesis in these crosses between *Triticum* and *A. elongatum*.

Discussion

There is considerable discrepancy between the observations and conclusions in this investigation and those reported by Vakar, Krot and Brekina (8) and Sapehin (6) on similar material. The former authors concluded that the amount of pairing in the F_1 of *A. elongatum* and *A. glaucum* when crossed with *Triticum* was similar, 10 bivalents on the average being formed. It is inconceivable that these observations can be accurate in view of the results obtained in the present investigation. Careful examination of the camera lucida drawings by Vakar, Krot and Brekina suggests that they have misinterpreted certain meiotic stages. For example their Figs. 6 and 8 do not appear typical heterotypic metaphase plates as named but rather to be in the anaphase stage with univalents splitting at the equatorial plate and with one polar group of chromosomes missing. Further, these authors include one typical side view of metaphase in *T. vulgare* × *A. glaucum* which clearly shows 7 bivalents, in agreement with the writer's observations. However, the majority of their observations were apparently made on polar views where it is extremely difficult to interpret the configurations.

Sapehin (6) evidently used a biotype of *A. elongatum* with 49 chromosomes so that his observations on crosses involving this species are not comparable with those made in the present study. He reports the presence of 2–3 bivalents in the F_1 of *T. vulgare* × *A. glaucum* whereas our cross between Kharkov × *A. glaucum* possessed an average of 5.6 bivalents and C.A.N. 1835 × *A. glaucum* possessed only 1.2 bivalents.

Tzitzin (7) and Vakar, Krot and Brekina (8) concluded that their morphological and cytological evidence indicated that *A. elongatum* and *A. glaucum* should be included in the genus *Triticum* rather than the genus *Agropyron*. These conclusions appear to be scarcely justified at present, since these species do not exhibit a higher degree of homology on crossing with *Triticum* than does *Aegilops* (1). An analogous case is found in *Lolium* and *Festuca*, the relative taxonomic positions of which have not been called in question in spite of the fact that the author (5) has shown that there is complete pairing of the parental complements in the F_1 hybrids of *Lolium perenne* × *Festuca*

pratensis. Cytological demonstration of genetic homology between primar sets of chromosomes or individual chromosomes in wide crosses does not i itself justify changing their taxonomic position. When the genetic relation of the various species of *Agropyron* to one another have been determine through hybridizing and subsequent meiotic studies, and when the relatio of the various *Agropyron* species to other members of the tribe has also bee determined, it should be possible to arrive at definite conclusions regardin the taxonomic position of *A. elongatum* and *A. glaucum*.

In two of the hybrids studied, the frequency of pairing was much lowe than that found in similar crosses or other collections of the same cross. In these instances either the homology of the parental chromosomes was different or else the homology remained unchanged but genes or gene complexes limited the degree of zygotene pairing. Several investigators (*e.g.*, Darlington, (3) have reported cases which indicated a segregation for genes limiting pairing. A similar case was reported in intergeneric hybrids between *Lolium perenne* and *Festuca pratensis* by the author (5) where five plants of a backcross gave chiasma frequencies per bivalent between 1.57 and 1.80 while two plants gave only 0.80 and 0.62. The same explanation would be reasonable for the F_1 of C.A.N. 1835 × *A. glaucum* in which there was an average of only 1.3 configurations per nucleus, whereas similar crosses involving Lutescens and Kharkov gave an average of 5.4 and 5.6 configurations per nucleus. C.A.N. 1835 is, however, a sixth generation hybrid between *T. durum* var. Pentad and *T. vulgare* var. Marquis and consequently a considerable number of structural changes may have altered the chromosomes to such an extent that their pairing behavior in wide crosses would be altered considerably.

The other example of an abnormally low degree of chromosome association was found in Collection 2 of Vernal × *A. elongatum*. In this case the *Triticum* parent was not in itself produced by hybridization. Intravarietal genetical differences in either of the parents would most likely be responsible and differences of this order would more likely be due to gene rather than structural changes. Therefore, at present it appears that this is another instance of genic limitation of pairing. Additional biotypes of these parents will have to be inter-crossed and the pairing behavior of the F_1 observed, before it will be possible to determine definitely the genetical basis for this abnormal behavior.

In spite of the irregularities described above, the normal behavior of the male parents and remaining crosses makes it possible to develop some conception of the genetic constitution and phylogeny of *A. glaucum* and *A. elongatum* and to obtain some information on the genetic relation of *Agropyron* and *Triticum*. This can only be accomplished if the primary sets of chromosomes are dealt with as a unit. Due allowance, however, must be made for the possibility of structural changes altering the pairing behavior of individual chromosomes. This method has already been used with fair success by numerous workers in determining the phylogenetic relation of the genera *Triticum*, *Aegilops*, *Secale* and *Haynaldia* of the tribe *Hordeae* (Aase, 1).

A limited amount of interchange between non-homologous chromosomes in *A. glaucum* has apparently occurred as shown by the presence of quadrivalents in this species and trivalents in the F_1 of crosses with tetraploid and hexaploid species of *Triticum*. The presence of approximately 5–6 bivalents in all but one of the above crosses indicates that either Set A or B of *Triticum* is partially homologous with one of the sets present in *A. glaucum*. Crosses will be made this season between *T. monococcum* and *A. glaucum* to determine which of these two sets is involved. In the meantime to simplify subsequent discussion the homologous set observed will be arbitrarily designated as A. If we designate the other two unidentified *Agropyron* sets as X and Y, then the hypothetical constitution of *A. glaucum* will be AXY.

In *A. elongatum* the situation is very complicated because of the high polyploid condition ($2n = 70$) and the large numbers of multivalent associations observed at meiosis. In spite of the presence of so many multivalent associations, it is unlikely that this species is a true autopolyploid. Numerically it is impossible for this 70-chromosome type to have arisen from repeated doubling of primary sets without hybridization having played some role. It is concluded, therefore, that this species probably arose through crossing of a hexaploid with a tetraploid species which would give rise to an unstable form with 35 chromosomes. The chromosome number in this unstable form would then become doubled to give *A. elongatum* with 70 chromosomes.

The number of univalents, bivalents and multiple configurations found in both *A. elongatum* and its crosses on *Triticum* give us certain clues as to the genetical constitution of *A. elongatum*. A large number of possible constitutions for *A. elongatum* were fitted to the data but only two appear feasible. The first and most promising explanation is that an *A. glaucum*-like hexaploid plant (AXY) was crossed with a tetraploid *Agropyron* of the constitution XY, *viz.*, AXY × XY = AXXYY ($2n = 35$). On doubling of this intermediate form a decaploid species such as *A. elongatum* would result. As pointed out previously, it is a simple matter to check the identity of set A. Additional crosses of *A. elongatum* with other members of the tribe Hordeae of known constitution should demonstrate whether or not X or Y are identical with any of the eight primary sets now identified (Aase, 1).

The pairing expectations from an AXXYY plant on selfing would be 7 bivalents of AA constitution, 7 quadrivalents of XXXX constitution and 7 quadrivalents of YYYY constitution or a total of 7 bivalents and 14 quadrivalents. The actual proportions found differed from the expected by the presence of an excess of bivalents and too few quadrivalents. Fewer quadrivalents may be explained by the fact that competition of pairing frequently results in four homologues forming two bivalents instead of one quadrivalent, and also by the occurrence of interchanges between X and Y which would increase the differentiation between pairs of chromosomes. Interchanges of this kind would also account for the presence of configurations of a higher valency than four. A single exchange of chromatin between X and Y in a plant of the constitution AXXYY could result in a ring of eight being formed and several of these have been observed in *A. elongatum*.

If a plant of the constitution AXXYY were crossed with *T. vulgare* of th known constitution ABC, *viz.*, ABC × AXXYY = AAXXYYBC ($2n = 56$) 14 univalents and 21 bivalents would be expected. Comparing this with th reported frequencies for the *T. vulgare* crosses in Table I, it will be seen tha the agreement for univalents is satisfactory while the actual number of bivalents is much less than expected and there is a proportional excess of configurations of a higher valency. While differentiation between pairs within four homologues in the *A. elongatum* parent would reduce the number of quadrivalents observed, because of competition of pairing, the same factors which caused this differentiation (*i.e.*, interchange between X and Y) would be expected to increase the number of multiple configurations in the F_1 of hybrids with *Triticum*. Since there is ample evidence that interchange has occurred frequently, the apparent discrepancies between the expected and observed number of configurations would be satisfactorily explained. The striking similarity between the numbers of trivalents, quadrivalents and quinquivalents observed in the crosses with different varieties of *T. vulgare* indicates that the structural differentiation involved was confined to the *A. elongatum* parent.

If a plant of the constitution AXXYY were crossed with *T. dicoccum* (AB), seven univalents would be expected and this agrees well with the observed frequency of 6.6 for Collection 1 of Vernal × *A. elongatum*. The number of bivalents and configurations of a higher valency are similar to those observed in the *T. vulgare* crosses and their occurrence can be explained in the same manner.

The alternative explanation of the constitution of *A. elongatum* is that it arose through the crossing of a hexaploid *Agropyron* species of the constitution ABY with a tetraploid wheat of the constitution AB, *viz.*, AB × ABY = AABBY ($2n = 35$). On doubling of this intermediate form, a decaploid species would result. The latter, on selfing, should form 7 bivalents and 14 quadrivalents, which is similar to the expectations for the alternative constitution AXXYY.

If a plant of the constitution AABBY was crossed with *T. vulgare* ABC, *viz.*, ABC × AABBY = AAABBBCY ($2n = 56$), 14 univalents and 14 trivalents would be expected. Again we find a close approximation between the expected and observed number of univalents. However, there are present less than one-third of the expected number of trivalents with all the bivalents and multiple configurations unaccounted for. In triploid species it is common to find only two of the three homologues pairing. If this occurred in these hybrids then the observed number of univalents would be expected to be much larger than observed. The occurrence of multiple configurations higher than three would again be accounted for by occasional interchanges. A partial test of the relative validity of these alternatives will be afforded this season by crossing *A. glaucum* with *A. elongatum*. If *A. elongatum* is of the constitution AXXYY, then no univalents should be present in the F_1; however, if its constitution is AABBY, 7 univalents should be present.

The first explanation of the origin and constitution of *A. elongatum* is the more attractive for the following reasons: (1) An interspecific cross is more likely to occur in nature than an intergeneric cross. (2) It seems unlikely that a hexaploid *Agropyron* species already exists with two wheat-like sets of chromosomes A and B. (3) From the morphological similarities of *A. glaucum* and *A. elongatum*, it is not unreasonable to suppose that one arose from the other through hybridization and subsequent chromosome doubling.

Acknowledgments

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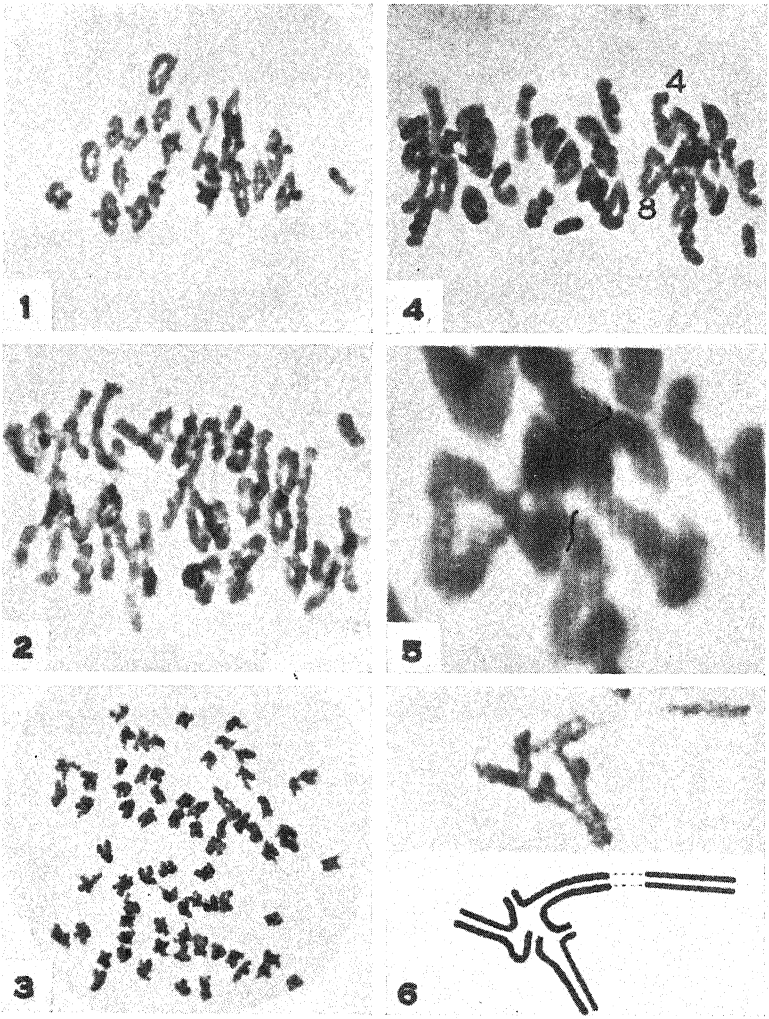

FIG. 1. *A. glaucum, 2 × I, 20 × II, × 1000.* FIG. 2. *A. elongatum, 2 × I, 19 × II, 6 × IV, 1 × VI, × 1000.* FIG. 3. *A. elongatum, heterotypic anaphase, 70 chr., × 650.* FIG. 4. *A. elongatum, 2 × I, 22 × II, 4 × IV, 1 × VIII, × 650.* FIG. 5. *A. elongatum, octavalent from Fig. 4, × 1790.* FIG. 6. *A. elongatum, diakinesis, octavalent and diagram showing interpretation. × 1140.*

NOTE.—*Roman numerals indicate valency of configuration, thus 2 × I = 2 univalents, etc.*

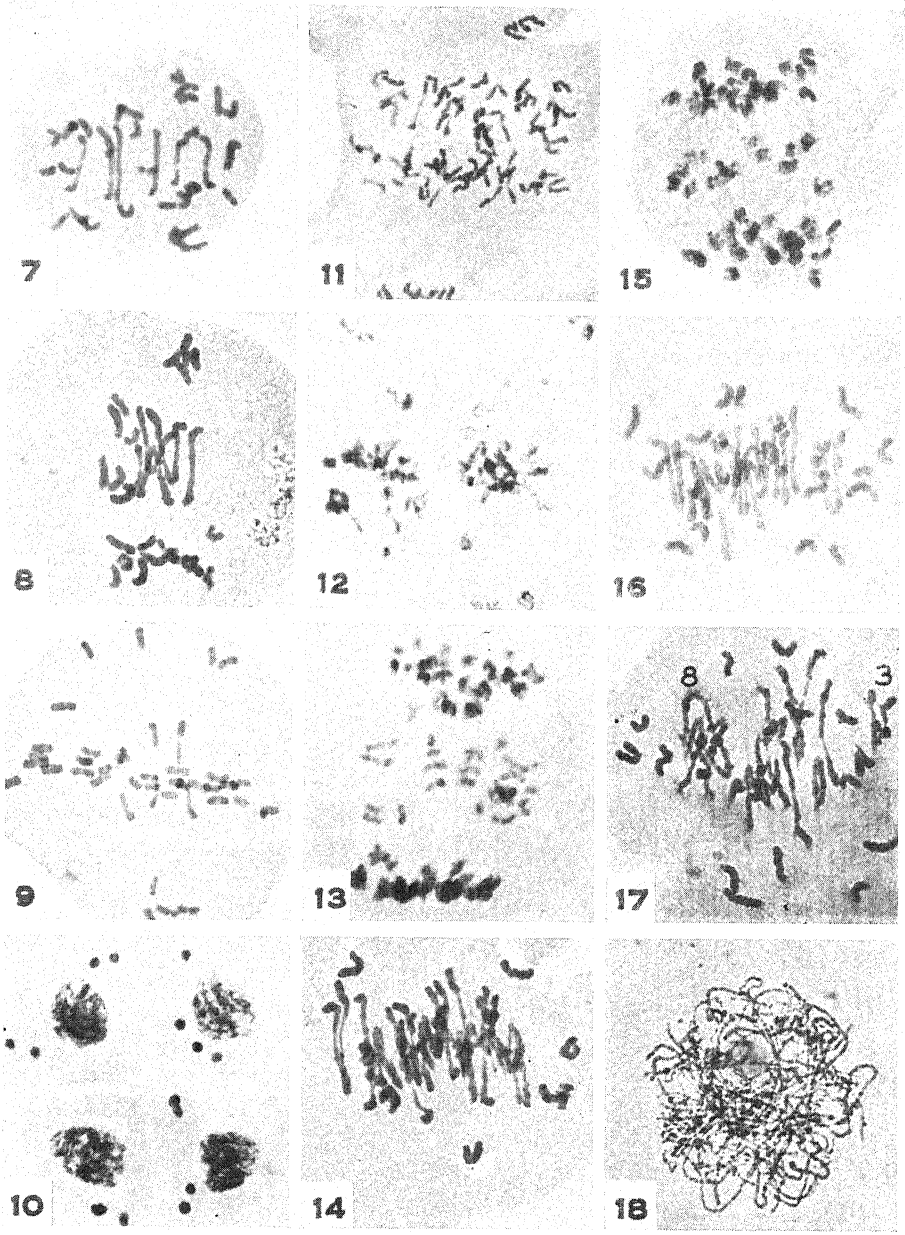

FIG. 7. *Lutescens* × *A. glaucum, metaphase, 28* × *I, 7* × *II.* FIG. 8. *Mindum* × *A. glaucum, metaphase, 29* × *I, 2* × *II, 3* × *III.* FIG. 9. *C.A.N. 1835* × *A. glaucum, metaphase, 42* × *I.* FIG. 10. *Emmer* × *A. elongatum, tetrad containing micronuclei.* FIG. 11. *Emmer* × *A. elongatum, homotypic anaphase.* FIG. 12. *Emmer* × *A. elongatum, homotypic metaphase.* FIG. 13. *Kharkov* × *A. elongatum, heterotypic anaphase, univalents splitting.* FIG. 14. *C.A.N. 1835* × *A. elongatum, 10* × *I, 10* × *II, 4* × *III, 1* × *IV, 2* × *V.* FIG. 15. *C.A.N. 1835* × *A. elongatum, heterotypic anaphase, 12 univalents splitting.* FIG. 16. *Lutescens* × *A. elongatum, 15* × *I, 10* × *II, 1* × *III, 2* × *IV, 2* × *V.* FIG. 17. *Lutescens* × *A. elongatum, 13* × *I, 10* × *II, 5* × *III, 1* × *VIII.* FIG. 18. *Lutescens* × *A. elongatum, leptotene stage. Magnifications of Figs. 7–18,* × *650.*

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THE BEHAVIOR OF PAIRED MONOSPOROUS MYCELIA OF *LENZITES SAEPIARIA* (WULF.) FR., *L. TRABEA* (PERS.) FR., *L. THERMOPHILA* FALCK, AND *TRAMETES AMERICANA* OVERH.[1]

BY IRENE MOUNCE AND RUTH MACRAE[2]

Abstract

Lenzites saepiaria, *L. trabea*, and *Trametes americana* are heterothallic and bipolar. In each species complete interfertility exists between haploid mycelia derived from different sources. *T. americana* is sometimes considered to be a pored form of *L. saepiaria*. The failure to obtain clamp connections in any pairing of a haploid mycelium of *L. saepiaria* with a haploid mycelium of *T. americana* seems significant and lends weight to the conclusion that these two forms are distinct. Haploid mycelia of *L. thermophila* are completely interfertile with those of *L. trabea*.

Introduction

Lenzites saepiaria (Wulf.) Fr. and *Trametes americana* Overh. (3) are two species of Polyporaceae which are found commonly on the wood of coniferous trees. The latter is the fungus which until recently has been referred to *T. odorata* (Wulf.) Fr. or to *T. protracta* Fr. by American authors. They resemble one another in shape and in the rusty brown color of pileus and context, but differ in that the hymenial region of *L. saepiaria* is typically lamellate (Plate I, 5) while that of *T. americana* is typically pored (Plate I, 7), although intergrading forms do occur. They are regarded usually as two distinct species, but it has been suggested that *T. americana* is but a pored form of *L. saepiaria*. *L. trabea* is distinct in the more grayish brown color of its context and of its hymenial surface, which is never entirely gilled but shows some elongate pores (Plate I, 1, 2, 3). It usually occurs on the wood of deciduous trees and all three are found on structural timbers.

Snell, Hutchinson and Newton (5) have shown by experiments with cultures of *T. americana (T. protracta)* and of *L. saepiaria* that, although their optimum temperature of growth is about the same, there is such a decided difference in both their upper limits of growth and their rates of growth that a test upon a single agar at temperatures of from 30° C. to 36° C. would serve to distinguish these two fungi in culture.

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Because of the similarities which do exist, we were interested in studying the behavior of paired monosporous mycelia of *L. saepiaria*, *L. trabea*, and *T. americana* and in applying to them the clamp-connection criterion for identity of species (6). Preliminary reports have been published in the Report of the Dominion Botanist for 1928, 1929, and 1930.

Isolation of Single Spores

Spores from a moistened fruit-body were allowed to fall directly on sterile lactose-dextrose gelatine in Petri dishes or a spore deposit was obtained from a sporophore produced in culture, mixed with a large drop of sterile distilled water, and smeared over the surface of the sterile gelatine. After germination, isolations were made by cutting out with a fine needle under the compound microscope a square of gelatine containing a single spore and placing this in a tube of potato-dextrose agar.

Paired Monosporous Mycelia

Lenzites saepiaria

Single basidiospore cultures were obtained from the sources indicated in Table I.

TABLE I

Culture No.	No. of isolations	Host	Locality
835	23	*Abies balsamea*	Timagami, Ont.
854	19	*Pinus Strobus*	Timagami, Ont.
996	14	*Pinus Strobus*	Cranberry Lake, Forest Camp, N.Y.
1655	10	*Pinus Strobus*	Meach Lake, P.Q.
1679	15	*Pseudotsuga taxifolia*	Coombs, B.C.
3250	8	*Picea alba*	Chalk River, Ont.
3252	14	*Pinus Banksiana*	Chalk River, Ont.

No clamp connections were found on any mycelium arising from a single basidiospore of *L. saepiaria* or of *T. americana*, but oidia were sometimes abundant. Falck (2) has given a detailed account of their development in *L. saepiaria*.

While working at this laboratory Miss D. N. Head isolated sixteen single oidium cultures of *L. saepiaria* and nine of *T. americana*. These mycelia were used in a large number of pairings but, as was to be expected, they behaved in exactly the same way as the haploid mycelium on which they developed, so that the results need not be given here in detail.

Pairings of monosporous mycelia were made on potato-dextrose agar in Petri dishes. The cultures were incubated for from ten to fourteen days, then examined under the microscope. The results of a series of all possible pairings of ten haploid mycelia of culture No. 996 are shown in Table II where the plus sign indicates the presence of clamp connections and the minus sign their absence.

TABLE II

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Lenzites saepiaria* No. 996

		A				a					
		1	4	6	8	2	3	5	7	9	10
A	1	−	−	−	−	+	+	+	+	+	+
	4	−	−	−	−	+	+	+	+	+	+
	6	−	−	−	−	+	+	+	+	+	+
	8	−	−	−	−	+	+	+	+	+	+
a	2	+	+	+	+	−	−	−	−	−	−
	3	+	+	+	+	−	−	−	−	−	−
	5	+	+	+	+	−	−	−	−	−	−
	7	+	+	+	+	−	−	−	−	−	−
	9	+	+	+	+	−	−	−	−	−	−
	10	+	+	+	+	−	−	−	−	−	−

TABLE III

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Lenzites saepiaria* No. 1679

		A				a					
		1	6	8	10	2	3	4	5	7	9
A	1	−	−	−	−	+	+	+	+	+	−
	6	−	−	−	−	+	+	−	+	+	+
	8	−	−	−	−	+	+	−	−	−	+
	10	−	−	−	−	+	−	−	−	−	+
a	2	+	+	+	+	−	−	−	−	−	−
	3	+	+	+	−	−	−	−	−	−	−
	4	+	−	−	−	−	−	−	−	−	−
	5	+	+	−	−	−	−	−	−	−	−
	7	+	+	−	−	−	−	−	−	−	−
	9	−	+	+	+	−	−	−	−	−	−

Similar series of pairings were made with haploid mycelia from cultures No. 835, 854, and 1655. In each case the haploid mycelia could be divided into two groups. Clamp connections were formed in every pairing of a member of one group with a member of the other group. *Lenzites saepiaria* is then heterothallic and bipolar. With culture No. 1679 the results obtained were less uniform as is shown in Table III. This might be accounted for, in part, by the fact that the spores were obtained from a sporophore which was moistened and revived after having been kept in the laboratory for two and a half years. However, even here, the results would indicate that the fungus is heterothallic and bipolar.

Pairings of haploid mycelia from different sources were made as indicated in Table IV.

TABLE IV

Culture No.	Host	Locality	Paired with	Culture No.	Host	Locality
835	*Abies balsamea*	Timagami, Ont.	×	854	*Pinus Strobus*	Timagami, Ont.
835	*Abies balsamea*	Timagami, Ont.	×	996	*Pinus Strobus*	Cranberry Lake Camp, N.Y.
996	*Abies balsamea*	New York	×	1655	*Pinus Strobus*	Meach Lake, P.Q.
3250	*Picea alba*	Chalk River, Ont.	×	3252	*Pinus Banksiana*	Chalk River, Ont.

In a total of 63 pairings, clamp connections were formed in every pairing and Table V is typical.

TABLE V

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Lenzites saepiaria* No. 835 WITH FIVE MONOSPOROUS MYCELIA OF *L. saepiaria* No. 854

854 \ 835	11	12	13	14	15
11	+	+	+	+	+
12	+	+	+	+	+
14	+	+	+	+	+
15	+	+	+	+	+
16	+	+	+	+	+

TABLE VI

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Trametes americana* No. 903

		A					a				
		1	4	8	10	11	2	3	5	6	7
A	1	−	−	−	−	−	+	+	+	+	+
	4	−	−	−	−	−	+	+	+	+	+
	8	−	−	−	−	−	+	+	+	+	+
	10	−	−	−	−	−	+	+	+	+	+
	11	−	−	−	−	−	+	+	+	+	+
a	2	+	+	+	+	+	−	−	−	−	−
	3	+	+	+	+	+	−	−	−	−	−
	5	+	+	+	+	+	−	−	−	−	−
	6	+	+	+	+	+	−	−	−	−	−
	7	+	+	+	+	+	−	−	−	−	−

TABLE VII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Trametes americana* No. 990 WITH FIVE MONOSPOROUS MYCELIA OF *T. americana* No. 903

903 \ 990	1	2	6	7	8
2	+	+	+	+	+
4	+	+	+	+	+
6	+	+	+	+	+
8	+	+	+	+	+
10	+	+	+	+	+

Therefore, complete interfertility obtained when pairings were made between haploid mycelia derived from fruit-bodies collected in different localities.

Trametes americana

Single basidiospore cultures were isolated from the sources shown in Table VIII.

TABLE VIII

Culture No.	No. of isolations	Host	Locality
903	10	*Picea canadensis*	Timagami, Ont.
990	8	*Thuja plicata*	Slocan Valley, B.C.
3251	18	*Picea alba*	Chalk River, Ont.
3253	14	*Pinus Banksiana*	Chalk River, Ont.
3254	8	*Pinus Banksiana*	Chalk River, Ont.

Like *L. saepiaria* this fungus is heterothallic and bipolar (Table VI). Haploid mycelia from different sources are mutually interfertile (Table VII) as shown by the results obtained from the pairings of such mycelia given in Table IX.

TABLE IX

Culture No.	Host	Locality	Paired with	Culture No.	Host	Locality
903	*Picea canadensis*	Timagami, Ont.	×	990	*Thuja plicata*	Slocan Valley, B.C
3251	*Picea alba*	Chalk River, Ont.	×	3253	*Pinus Banksiana*	Chalk River, Ont.
3251	*Picea alba*	Chalk River, Ont.	×	3254	*Pinus Banksiana*	Chalk River, Ont.

In a total of 44 pairings, clamp connections were formed in every pairing (Table VII). These results show clearly that both *L. saepiaria* and *T. americana* are heterothallic and bipolar and in each species complete interfertility exists between haploid mycelia derived from different sources.

At Chalk River, Ontario, Mr. A. W. McCallum and Mr. C. G. Riley collected sporophores of *Lenzites saepiaria* No. 3250 and *Trametes americana* No. 3251 which were growing within three feet of each other on a *Picea alba* log, and sporophores of *L. saepiaria* No. 3252 and *T. americana* No. 3253 on the one *Pinus Banksiana* log. Single basidiospore cultures were isolated from one sporophore in each of these four collections and the pairings were made as shown in Table X.

TABLE X

Culture No.	Fungus	Paired with	Culture No.	Fungus	No. of pairings	Clamp connections	
						Present	Absent
3250	*L. saepiaria* from *Picea*	×	3252	*L. saepiaria* from *Pinus*	8	8	0
3251	*T. americana* from *Picea*	×	3253	*T. americana* from *Pinus*	10	10	0
3250	*L. saepiaria* from *Picea*	×	3251	*T. americana* from *Picea*	8	0	8
3250	*L. saepiaria* from *Picea*	×	3253	*T. americana* from *Pinus*	10	0	10
3252	*L. saepiaria* from *Pinus*	×	3251	*T. americana* from *Picea*	10	0	10
3252	*L. saepiaria* from *Pinus*	×	3253	*T. americana* from *Pinus*	10	0	10

Haploid mycelia of *L. saepiaria* from *Picea* were completely interfertile with those of *L. saepiaria* from *Pinus;* haploid mycelia of *T. americana* from *Picea* were completely interfertile with those of *T. americana* from *Pinus*, but clamp connections were not formed in any of the 38 pairings of a haploid mycelium of *L. saepiaria* with a haploid mycelium of *T. americana*. In view of the complete interfertility obtained in all our pairings of haploid mycelia of *T. americana* from different sources and similar interfertility between haploid mycelia of *L. saepiaria* from different sources the failure to obtain clamp connections in any pairing of a haploid mycelium of *T. americana* with a haploid mycelium of *L. saepiaria* seems significant and lends weight to the conclusion of Snell (6) and others that these two forms are distinct.

Lenzites trabea and *L. thermophila*

Of these two fungi Mr. Cartwright (1) writes as follows: "This fungus (*L. trabea* from Dr. Audrey Richards) has been compared with a culture of *L. thermophila* Falck from the Centraalbureau voor Schimmelcultures, Baarn,

with one received direct from Falck and also with a culture kindly sent to us by Dr. Liese of *Trametes protracta*. The four cultures are identical in every respect." It seemed an interesting case in which to apply the clamp-connection criterion for identity of species.

Single basidiospore cultures were isolated from the following sources:

L. trabea No. 3237. Sporophores which developed on a bench made of *Taxodium distichum* in a greenhouse, Arboretum, Experimental Farm, Ottawa. (Plate I, 3).

L. trabea No. 1681. A culture received from Mr. Cartwright, Princes Risborough, England, and sent to him by Dr. Audrey Richards of the Forest Products Laboratory, Madison, Wis.

L. thermophila No. 1682. A culture received from Mr. Cartwright.

Series of all possible pairings of monosporous mycelia of *L. trabea* No. 1681 and No. 3237 and of *L. thermophila* No. 1682 showed that each fungus was heterothallic and bipolar (Table XI).

TABLE XI

THE RESULTS OF PAIRING IN ALL POSSIBLE COMBINATIONS TEN MONOSPOROUS MYCELIA OF *Lenzites trabea* No. 3237

		A							a		
		1	6	9	10	15	16	18	2	7	12
A	1	−	−	−	−	−	−	−	+	+	+
	6	−	−	−	−	−	−	−	+	+	+
	9	−	−	−	−	−	−	−	+	+	+
	10	−	−	−	−	−	−	−	+	+	+
	15	−	−	−	−	−	−	−	+	+	+
	16	−	−	−	−	−	−	−	+	+	+
	18	−	−	−	−	−	−	−	+	+	+
a	2	+	+	+	+	+	+	+	−	−	−
	7	+	+	+	+	+	+	+	−	−	−
	12	+	+	+	+	+	+	+	−	−	−

TABLE XII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Lenzites trabea* No. 1681 WITH THREE MONOSPOROUS MYCELIA OF *L. thermophila* No. 1682

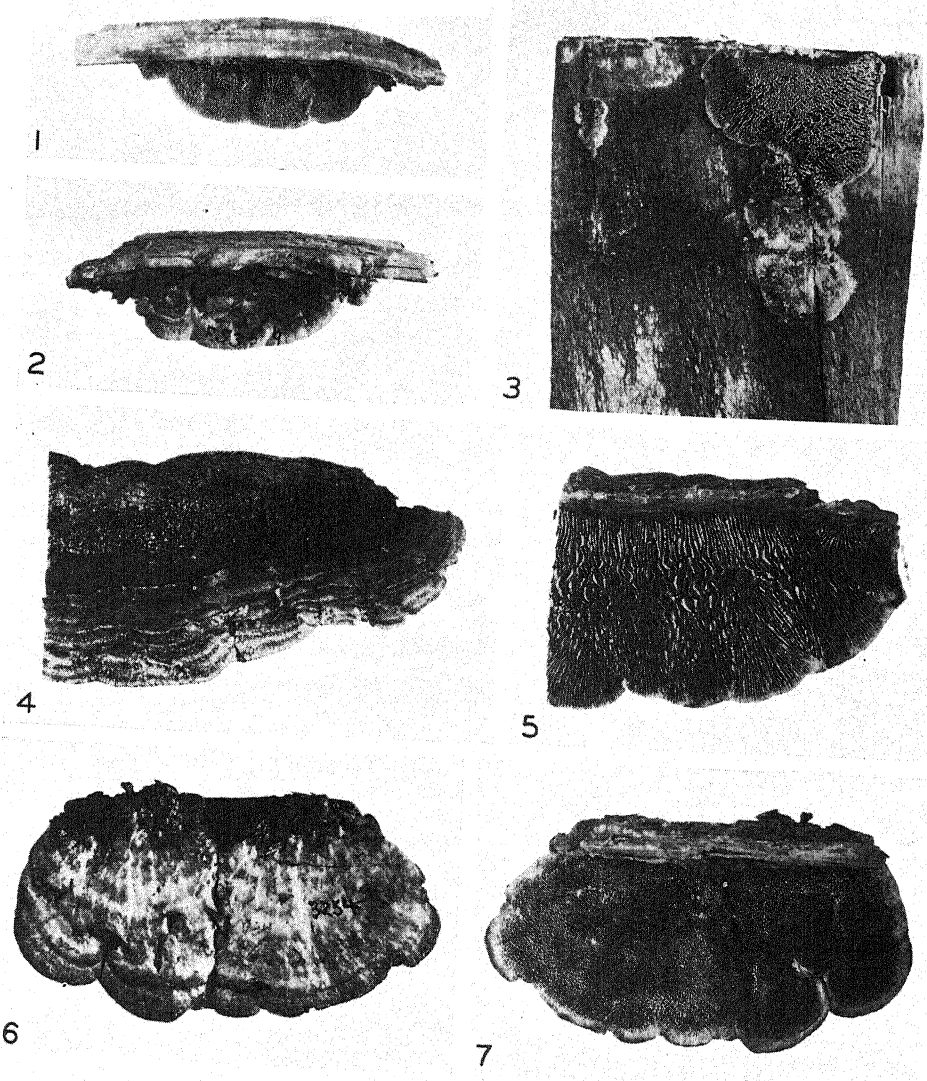
		1681				
		1	2	3	4	5
1682	1	+	+	+	+	+
	2	+	+	+	+	+
	3	+	+	+	+	+

TABLE XIII

THE RESULTS OF PAIRING FIVE MONOSPOROUS MYCELIA OF *Lenzites trabea* No. 3237 WITH TWO MONOSPOROUS MYCELIA OF *L. thermophila* No. 1682

		3237				
		1	2	3	4	5
1682	2	+	+	+	+	+
	5	+	+	+	+	+

Haploid mycelia of *L. trabea* were paired with haploid mycelia of *L. saepiaria* and *T. americana* from various sources, but though 109 pairings were made, no clamp connections were formed in any pairing; but clamp connections were formed in every pairing of a haploid mycelium of *L. trabea* No. 1681 and *L. trabea* No. 3237 showing that they were mutually interfertile, and in every pairing of a haploid mycelium of *L. thermophila* with a haploid mycelium from either of the *L. trabea* cultures (Tables XII and XIII). This would indicate that by the clamp-connection criterion *L. trabea* and *L. thermophila* No. 1682 are one and the same species, and corroborates Mr. Cartwright's conclusion.



FIGS. 1–2. *Lenzites trabea No. 5369 showing hymenial surface and pileus.* FIGS. 3–7. *Sporophores from which spores for experiments were obtained.* FIG. 3. *L. trabea No. 3237.* FIGS. 4–5. *Upper and lower surface of L. saepiaria No. 3250.* FIGS. 6–7. *Upper and lower surface of Trametes americana No. 3254. Three-fourths natural size.*

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THE EFFECT OF FERMENTATION ON SOME PROPERTIES OF GLUTEN[1]

By G. H. Guest[2] and R. K. Larmour[3]

Abstract

Except in the cases of a flour of unusually high protein content (19%) and a Pacific Club wheat flour of 7.6% protein, the loss of gluten protein after four hours' fermentation was about 17% and the change in water-holding capacity was negligible. There was practically no differentiation between flours from heavily frosted wheat and average sound flours. The 19% protein flour, however, showed a loss of gluten protein of only 5% and a slight increase in water-holding capacity; the Club flour showed a loss of 43.4% in gluten protein and a decrease of 27% in water-holding capacity. It is concluded that these two properties of gluten cannot be used to differentiate most flours.

Introduction

During the past decade numerous studies of the relation of wheat and flour protein to baking strength have confirmed the conclusion reached by Zinn (9) in 1923 that these characteristics are significantly and positively correlated. It is unnecessary to discuss again in detail these various investigations; suffice it to state that many workers have concluded, with remarkable unanimity, that protein content of wheat or flour is at present the best single-figure estimate of baking strength. Blish and Sandstedt (2) in discussing the term "flour strength" state that "for all practical purposes, protein content and inherent flour strength are one and the same thing." By the term "flour strength" they refer to the potentialities of the flour rather than to its behavior in any specific baking procedure.

In spite of the general conviction that protein content furnishes a reliable criterion of strength of sound wheat, there is some reluctance to abandon the baking test and it is still common practice to use the results of this test in the final estimate of strength. Many other methods, less time-consuming and presumably more amenable to accurate measurement, have been devised and tried with indifferent success. Of these there might be mentioned the various types of extensimeters, the instruments designed to measure resistance of doughs to mixing, the dough-ball fermentation test, the viscosity test of acidulated flour-water suspensions, and the washed-gluten test. All of these have been devised with a view to getting a figure or figures with which to predict how the flour is likely to behave when subjected to the baking procedure.

The washed-gluten test is the oldest of the short methods of appraising flour characteristics, and it requires the least equipment. There is an exceedingly high positive correlation between dry gluten and protein, as determined by the Kjeldahl method, and if it were as accurate as the latter it would

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be a superior method, because one can determine not only the quantity, bu also to some extent the quality, from consideration of the "feel" of the glute mass as it is being worked from the dough. This test is very useful whe applied by a skilled technician, but unfortunately it is difficult to standardiz the procedure sufficiently to achieve good agreement among the results ob tained by various individuals. This, however, is not sufficient reason fo discarding it altogether. The Kjeldahl test, while very accurate, often lead to inaccurate conclusions. Flours of the same protein content, which migh be predicted to possess the same baking characteristics, frequently prove quite diverse. Those that fall below expectation are considered of poor quality. In this connection Blish and Sandstedt (2) have made the excellent suggestion that the term "weak" should be taken to indicate the inability of a flour to bake to the standard predicted from its protein content. If a flour fails, when tested by all available means, to come up to the expectations based on its protein content, it must be assumed that the protein has been damaged by adverse conditions of climate or of storage after harvesting, or that it is inherently inferior. In either case prediction equations applicable to normal protein would be invalid.

The weakness might be attributed to inability of the protein to absorb and hold enough water, or the converse, or to a tendency to disperse too readily during fermentation, or to many other causes. In any case, the fact is that the weak flour responds differently to the effect of fermentation than the normal flour of the same protein content. If one were interested in gaining more information regarding the differences between weak and normal flours, it would seem logical to examine the gluten before and after fermentation to determine whether some measurable characteristic was capable of providing an explanation for the differences in baking behavior.

Several workers have approached this problem along these lines. Sharp and Gortner (7) observed that the character of the gluten changed after the first hour of fermentation. Towards the end of the washing period the gluten ball showed a tendency to disintegrate and it was impossible to avoid losses of gluten from this cause. Although the quantity of gluten obtainable decreased, the hydration increased. After eight hours' fermentation no gluten could be separated by washing with distilled water, but if 1% sodium chloride solution was used, gluten could be isolated after 24 hours' fermentation. Sharp and Schreiner (8), using fractionation analysis of the gluten, were unable to find any marked changes resulting from fermentation. Johnson and Green (5) found that the viscosity of flour-water suspensions showed marked decrease as a result of fermentation. Brownlee and Bailey (3) observed that saline peptization of doughs fermented for various periods indicated no change in the proteins as fermentation progressed and concluded that no chemical changes had been effected. They suggested that the progressive increase in acidity as fermentation proceeds causes gradual changes in the dough that affect the imbibitional capacities, and this might account for the changes in viscosity observed by Johnson and Green (5).

The fact that solubility in various solvents appears to be unaffected by rmentation makes it seem unlikely that any important chemical change, ch as partial hydrolysis, occurs. On the other hand, if the idea of McCalla id Rose (6) that gluten is a protein complex rather than a mixture is correct, e failure to obtain gluten after fermentation would suggest some sort of ydrolysis. In the study herein reported attention was confined to two properes of gluten, namely, the quantity procurable and its water content.

Material and Methods

The flours selected for this investigation are described in Table I. They ere all experimentally milled except two, namely, No. 4, a hard red spring anadian commercial patent, and No. 5, a Pacific Club commercial patent. amples No. 17, 18 and 19 were procured by compositing experimentally illed flours from commercial samples of Grades No. 4 Northern, No. 5 and lo. 6.

TABLE I

DESCRIPTION AND ANALYSIS OF FLOURS USED

Flour No.	Origin	Moisture, %	Protein (13.5% moisture basis)	Protein (dry basis)
1	Saskatoon composite, Marquis	12.6	19.2	21.97
2	Saskatoon composite, Marquis	12.5	17.9	20.46
3	Saskatoon composite, Marquis	12.6	16.2	18.54
4	Commercial patent	12.9	13.7	15.73
7	Saskatoon composite, Marquis	12.3	13.0	14.82
11	Garnet, composite	12.2	13.2	15.03
16	Winnipeg composite, Marquis, Frosted, Grade 3	12.8	13.1	15.02
17	Winnipeg composite, Marquis, Frosted, Grade 4	12.7	13.4	15.35
78	Saskatoon composite, Marquis	12.1	11.9	13.55
18	Winnipeg composite, Marquis, Frosted, Grade 5	12.7	11.9	13.63
19	Winnipeg composite, Marquis, Frosted, Grade 6	12.8	11.7	13.42
5	Pacific Club	12.5	7.7	8.8

The glutens were washed from doughs containing 25 gm. flour, 5% yeast, 3% sugar and 1% sodium chloride. Washing was done by hand with tap water at 25° C. flowing at the rate of 110 cc. per minute. In all instances the washing time was 12 minutes. Before weighing, the gluten was thoroughly kneaded again, shaped into a small ball, and finally pressed between sheets of filter paper. While the method of Dill and Alsberg (4) is admitted to be superior to this, it is undoubtedly less convenient and, as only comparative results were desired, the simplest method was chosen. Further simplification of procedure was achieved by dispensing with immersion of the dough ball for one hour before washing and of the gluten for one hour after washing. Berliner and Koopmann (1) considered the former unnecessary, and in the present study it was impossible to use it except with unleavened doughs.

As a preliminary, one of us washed many glutens from a stock flour, until sufficient skill was achieved to replicate results on the same day and on different days. After that, the regular method, which involves immersion of dough and gluten, was compared with the proposed shorter method. The results of this comparison are given in Table II. They indicate that more uniform results are obtainable with the shorter method and that the average values by the two methods do not vary significantly.

TABLE II

WEIGHTS OF WET AND DRY GLUTEN OBTAINED FROM 25 GM. FLOUR No. 4 (DRY BASIS) IN 12 REPLICATIONS USING TWO DIFFERENT PROCEDURES

SHORT PROCEDURE			STANDARD PROCEDURE		
Experiment No.	Weight of wet gluten, gm.	Weight of dry gluten, gm.	Experiment No.	Weight of wet gluten, gm.	Weight of dry gluten, gm.
1	13.49	4.26	1	13.27	4.31
2	13.93	4.57	2	13.44	4.27
3	13.96	4.62	3	13.84	4.49
4	13.57	4.52	4	13.65	4.44
5	13.43	4.45	5	13.65	4.32
6	13.43	4.42	6	13.70	4.39
7	13.69	4.32	7	13.49	4.44
8	13.61	4.40	8	13.81	4.35
9	13.53	4.35	9	13.94	4.47
10	13.84	4.54	10	13.74	4.43
11	13.68	4.41	11	13.43	4.26
12	13.40	4.37	12	13.97	4.54
Total	163.56	53.23		163.93	52.71
Average	13.63 ± .0362	4.44 ± .0201		13.66 ± .0454	4.39 ± .0168
Range	13.40 – 13.96	4.26 – 4.62		13.27 – 13.97	4.26 – 4.54
Standard deviation, σ	.1861	.1034		.2082	.0865

The Effect of Fermentation on the Quantity of Gluten Protein

In Table III there are given the quantities of dry gluten obtained from doughs fermented for four hours at 30° C. and the checks which were obtained from similar doughs without fermentation. There are also given the protein contents of these glutens and various calculated values.

In all cases the actual quantity of dry gluten decreased as a result of fermentation. The dry gluten, however, is not a reliable figure on which to base comparisons because the protein content of glutens varies considerably, no matter what care is taken in the washing. A more reliable figure is the quantity of gluten protein. This value is obtained by multiplying the weight of dry gluten by its protein content as determined by the Kjeldahl method. For convenience this value has been expressed as percentage of flour. Decreases of this value resulting from the four-hour fermentation must be attributed to hydrolysis or to peptization of part of the gluten protein. From column 9, Table III, it can be observed that the percentage decreases were appreciable except with flour No. 1 which was of exceptionally high protein content.

TABLE III

E EFFECT OF FERMENTATION ON THE QUANTITY OF GLUTEN PROTEIN OBTAINED FROM TWELVE DIFFERENT FLOURS

ur).	Description	Fermentation time, hr.	Total protein in flour, (dry basis), (N ×5.7), %	Dry gluten, average of 3 det'ns from 25 gm. flour (dry basis), gm.	Dry gluten as % of flour	Protein in dry gluten (N ×5.7) average of 3 det'ns. %	Gluten protein as % of flour	Loss of gluten protein resulting from 4 hr. fermentation, %	Difference between protein in flour and gluten protein in flour expressed as % of total protein in flour
	Marquis	0	22.0	6.13	24.5	77.8	19.1		13.2
		4		5.26	21.0	86.2	18.1	4.9	17.5
	Marquis	0	20.5	5.69	22.8	79.8	18.2		11.2
		4		4.60	18.4	86.0	15.8	12.9	22.7
	Marquis	0	18.5	5.00	20.0	80.9	16.2		12.7
		4		3.93	15.7	86.6	13.6	15.9	26.6
	Commercial patent	0	15.7	4.21	16.8	77.2	13.0		17.4
		4		3.15	12.6	84.5	10.6	18.1	32.3
	Frosted 4°	0	15.4	4.04	16.2	83.0	13.4		12.6
		4		3.23	12.9	85.0	11.0	18.2	28.5
	Garnet	0	15.0	3.92	15.7	84.4	13.2		12.0
		4		3.28	13.1	84.0	11.0	16.6	26.6
	Frosted 3°	0	15.0	3.99	16.0	81.5	13.0		13.4
		4		3.11	12.4	85.4	10.6	18.3	29.2
	Marquis	0	14.8	4.07	16.3	80.8	13.2		11.3
		4		3.29	13.2	84.3	11.1	15.6	25.1
	Frosted 5°	0	13.6	3.45	13.8	83.5	11.5		15.5
		4		2.88	11.5	82.5	9.5	17.5	30.3
	Marquis	0	13.6	3.37	13.5	84.3	11.4		16.1
		4		3.01	12.0	81.1	9.8	14.1	27.9
	Frosted 6°	0	13.4	3.32	13.3	82.8	11.0		18.0
		4		2.89	11.6	82.3	9.5	13.4	29.1
	Pacific Club	0	8.8	2.10	8.4	79.6	6.7		24.0
		4		1.22	4.9	77.7	3.8	43.4	56.9

lour No. 5, from Pacific Club, gave an enormous decrease amounting to 3.4%. All the others gave decreases ranging from 12.9% to 18.3%. hese decreases do not appear to be closely associated with the strength of ıe flours. For instance, Nos. 78 and 19, of nearly the same protein content ut from wheat of very different commercial grades, gave practically the ame percentage decrease, although No. 19 was milled from wheat of grade Jo. 6, severely damaged by frost. Again flour No. 11, milled from Garnet heat, showed less dispersion than flour No. 4, the commercial hard red pring patent. The only important differentiation in this series in regard to ercentage of gluten protein dispersed as a result of fermentation is found with

flours No. 1 and 5. With flour No. 5 it was difficult to wash out the gluten after four hours' fermentation and the great decrease may therefore be accounted for in part as owing to loss in washing. This loss in washing is of course the result of some change in the protein during fermentation. This property of gluten does not seem to offer much promise as a means for differentiating flours of the same class.

The Effect of Fermentation on the Water-holding Capacity of Crude Gluten

The water contained in the glutens from leavened doughs before and after four hours' fermentation was calculated as a percentage on the basis of dry crude gluten and also dry gluten protein. These data, which are given in Table IV, show that in most cases there is not much change in water-holding capacity as a result of fermentation. Exceptions to this are seen in Flours 1,

TABLE IV

THE EFFECT OF FERMENTATION ON THE WATER-HOLDING CAPACITY OF THE GLUTEN PROTEIN

Flour No.	Description	Fermentation time, hr.	Protein in flour, dry basis, (N × 5.7), %	Gluten protein per 100 gm. flour	Weight of water in gluten from 100 gm. flour (dry basis), gm.	Water as % of crude, dry gluten	Water as % of gluten protein
1	Marquis	0	22.0	19.1	48.6	198	255
		4		18.1	49.5	235	273
2	Marquis	0	20.5	18.2	45.8	201	252
		4		15.8	39.0	212	247
3	Marquis	0	18.5	16.2	40.6	203	250
		4		13.6	32.5	207	239
7	Marquis	0	14.8	13.2	29.0	178	221
		4		11.1	24.7	188	222
78	Marquis	0	13.6	11.4	25.1	186	221
		4		9.8	21.2	176	217
16	Marquis, Frosted 3°	0	15.0	13.0	31.0	195	239
		4		10.6	24.3	196	229
11	Garnet	0	15.0	13.2	30.2	192	228
		4		11.0	24.0	183	217
17	Marquis, Frosted 4°	0	15.4	13.4	31.6	196	236
		4		11.0	25.6	199	234
18	Marquis, Frosted 5°	0	13.6	11.5	26.0	188	225
		4		9.5	21.0	183	222
19	Marquis, Frosted 6°	0	13.4	11.0	22.1	166	201
		4		9.5	19.2	166	201
4	Commercial Patent	0	15.7	13.0	34.9	207	269
		4		10.6	26.2	208	246
5	Pacific Club	0	8.8	6.7	18.8	224	281
		4		3.8	7.8	160	206

4 and 5. Flour 1, which was very high in protein, showed an increase, while Flours 4 and 5 showed decreases, the decrease in the latter being very great. It should be noted that the water-holding capacity of the Pacific Club flour before fermentation was higher than that of any of the spring wheat flours, and after fermentation it was lower than any of them except No. 19, which was from severely frosted wheat. It is also of interest that Flour 19 showed no change as a result of fermentation.

It seems evident that the water-holding capacity of glutens is little affected by fermentation except in a few cases. The exceptions noted in this study were (a) a flour of unusually high protein content, which showed an increase, and (b) a flour of very low protein content from Pacific Club wheat, which showed a very marked decrease as a result of four hours' fermentation. It was most astonishing to find that flours from severely frosted wheat, grading No. 5 and No. 6, showed no appreciable decrease in water-holding capacity after fermentation. It should be recalled, too, that these flours exhibited no greater loss of actual gluten than did flours from sound wheats. Of course, the glutens could be readily differentiated by "feel" but as there was no way of recording this property accurately it has not been taken into consideration in this discussion.

We are therefore forced to the conclusion that neither the quantity of gluten nor its water content can be used for differentiating many flours that by other experimental tests and by practical experience can be shown to be widely different in quality.

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WEATHER AND WHEAT YIELD IN WESTERN CANADA

II. INFLUENCE OF PRE-SEASONAL PRECIPITATION ON PLOT YIELDS
III. RELATION BETWEEN PRECIPITATION AND AGRICULTURAL YIELD[1]

By J. W. Hopkins[2]

Abstract

In continuance of a previous statistical study, the correlation between plot yields of wheat grown at experimental stations in central and southern Saskatchewan and Alberta and the amount of precipitation during the autumn, winter and spring months prior to sowing was investigated.

There was a significant relation between pre-seasonal precipitation and the yield secured from year to year on both the fallowed and stubble plots of a summerfallow-wheat-wheat rotation, above-average moisture being associated with increased yields. The annual yields of Marquis wheat from more fertile summerfallowed varietal test plots were not, however, significantly correlated with pre-seasonal precipitation, nor was there any consistent relation between this weather factor and the relative yield of certain early, medium-early and late-maturing varieties.

The annual average yield of wheat per acre from 1916–34 in three central and in three southern crop districts of Saskatchewan and Alberta showed a significant positive correlation with the available statistics of rainfall between May 1 and July 31. Yields in the southern districts were also positively correlated with pre-seasonal precipitation, whereas those in the central districts were not. The degree of association ($R = 0.74$, central; and 0.79, southern) was not adequate for the practical forecasting of annual production, but may be improved by refinements dependent on the accumulation of additional observational data.

Introduction

In a previous communication (8), the author reported a statistical study of the relation between annual variations in rainfall and temperature during the growing season and the yield of wheat from certain plots at agricultural experiment stations in central and southern Alberta and Saskatchewan. Three series of crop records were employed, permitting the correlation of the weather factors mentioned with (i) the yield of Marquis wheat grown in variety test plots; (ii) the relative order of yield of the wheat varieties Garnet (early maturing), Reward (early maturing), Marquis (medium-early) and Red Fife (late maturing); and (iii) the yield from the summerfallowed and stubble plots of a summerfallow-wheat-wheat rotation.

A significant relation between yield and the amount and distribution of seasonal rainfall was demonstrable from the first and third of these series. On the whole, above-average rainfall was associated with higher yield, though the result of a given increment of rain at a specified time seemed to be partly dependent on soil conditions. It was also concluded that the influence of weather conditions was not exerted mainly during a few "critical periods", but extended throughout the growing season.

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An appreciable proportion of the recorded variation in yield was thus accounted for, but there remained in all series large residual variations. Although these undoubtedly arose in part from the fact that, at each station, the crop was grown on different soil each year, it was felt that the amount of precipitation during the autumn, winter and spring months might also have appreciably affected crop growth. The investigation has therefore been amplified by the inclusion of this factor in each of the three series of crop and weather correlations. An account of the results thus obtained is given in the first part of the present paper.

Following these studies of plot data, some preliminary consideration is given to the problem of determining the relation between weather conditions and agricultural yields by crop districts. The ultimate objective here is the formulation of regression or prediction equations which will enable a reasonable estimate of the size of a forthcoming crop to be made from a knowledge of the weather conditions affecting its development.

II. Influence of Pre-seasonal Precipitation on Plot Yields

A. Total Pre-seasonal Precipitation (Rain and Snow)

The total amounts of precipitation recorded each season, from August 1 of the preceding year to the date sixteen days before the sowing of the crop, for the various stations and seasons for which yield data were available for the previous study (8)*, are shown in Table I. The figures given include both rain and snow, but are expressed as "inches of rain", the customary conversion factor of 1/10 having been used to obtain the rain equivalent of snowfall.

TABLE I

Pre-seasonal precipitation (rain and snow), expressed as inches of rain

Crop year	Edmonton	Lacombe	Lethbridge	Indian Head	Swift Current	Scott	Rosthern	Saskatoon*
1931	5.90	7.22	6.30	4.53	6.69	5.73	8.20	6.94
1930	5.71	5.80	8.37	8.90	5.34	5.29	7.32	4.23
1929	7.16	7.53	6.86	3.13	5.15	3.69	5.95	5.68
1928	8.30	12.01	13.43	11.32	9.11	7.76	6.57	7.47
1927	13.03	17.49	11.87	12.42	10.56	7.95	7.54	9.88
1926	8.35	14.17	9.98	7.51	7.64	6.79	8.78	6.89
1925	10.29	11.16	11.08	13.44	10.24	10.42	9.70	10.42
1922	5.88	4.96	7.42	13.99	—	4.36	6.92	7.45
1921	8.62	7.54	5.59	9.89	—	9.19	10.09	11.55
1920	11.75	10.79	12.63	11.85	—	8.98	8.27	8.89

* *University of Saskatchewan.*

In order to specify the average effect of variations in previous rain and snow on yield, the partial regression, after allowing for the influence of succeeding rainfall during the growing season, was determined in each case. Utilizing previous arithmetical working, the calculation of each of these coefficients was accomplished in three stages as follows:

* *Mr. J. Patterson, Director of the Meteorological Service of Canada, kindly co-operated in this connection by supplying records not available in published form.*

(i) The seasonal variance of pre-seasonal precipitation and its covariance with yield were first calculated directly from the observational data. (ii) Then the regression of pre-seasonal precipitation on the amount and distribution of rainfall during the growing season, as specified by the rainfall distribution coefficients of the previous paper (8), was determined. Knowing now the crude variance and covariance of yield and pre-seasonal precipitation, and the regression of both yield (8) and pre-seasonal precipitation on the rainfall coefficients for the growing season, the final step (iii) was to compute the variance and covariance of the yield and pre-seasonal precipitation residuals, and from these the partial regression of yield on pre-seasonal precipitation. Since the three series of crop records were of unequal length, it was necessary to follow through the complete procedure for each series separately.

Pre-seasonal Precipitation and Yield of Marquis Wheat

(i) The method of calculating the seasonal variance and covariance of the crop and meteorological data has already been explained (8, page 312). Varietal test plot yields of Marquis wheat for ten years at each of seven stations have also been published (8, Table I).

From the data in Table I of the present paper the sum of the squares of the seasonal deviations of the corresponding pre-seasonal precipitation from the station averages was found to be 499.3909. In the same way these and the foregoing data gave 633.8319 as the sum of the products of the annual fluctuations of yield and pre-seasonal precipitation.

(ii) The regression coefficients $\beta_0, \beta_1, \ldots \beta_5$ of pre-seasonal precipitation p on the distribution coefficients $\rho_0, \rho_1, \ldots \rho_5$ of rainfall during the growing season were determined from the Normal Equations given below, of which the numerical coefficients of the left-hand side are derived as previously (8, page 312), and those of the right-hand side are the sums of the products of the seasonal deviations of p and the rainfall distribution coefficients $\rho_0, \rho_1, \ldots \rho_5$ (8, Table IV) respectively.

$$
\begin{aligned}
16.91616\beta_0 + .704273\beta_1 - 7.374067\beta_2 - .153974\beta_3 \\
+ 3.366082\beta_4 + 2.677181\beta_5 = 42.56497 \\
.704273\beta_0 + 13.445682\beta_1 - .966389\beta_2 - 5.327921\beta_3 \\
+ .238760\beta_4 + 2.090910\beta_5 = -15.60284 \\
-7.374067\beta_0 - .966389\beta_1 + 15.382760\beta_2 + 4.591087\beta_3 \\
- 3.442486\beta_4 - 2.087548\beta_5 = -25.84757 \\
- .153974\beta_0 - 5.327921\beta_1 + 4.591087\beta_2 + 16.260811\beta_3 \\
+ .035470\beta_4 - 3.460086\beta_5 = 3.38522 \\
3.366082\beta_0 + .238760\beta_1 - 3.442486\beta_2 + .035470\beta_3 \\
+ 18.600138\beta_4 + .981074\beta_5 = 18.96055 \\
2.677181\beta_0 + 2.090910\beta_1 - 2.087548\beta_2 - 3.460086\beta_3 \\
+ .981074\beta_4 + 13.885332\beta_5 = 9.24139 \quad \text{(I)}
\end{aligned}
$$

These equations gave the following values for the unknowns:

$$\beta_0 = 2.16962 \qquad \beta_3 = .00902$$
$$\beta_1 = -1.37242 \qquad \beta_4 = .52178$$
$$\beta_2 = -.56712 \qquad \beta_5 = .33365$$

The corresponding regression coefficients α of yield on the rainfall coefficients were (8, p. 213)

$$\alpha_0 = 5.16387 \qquad \alpha_3 = .80204$$
$$\alpha_1 = -3.74079 \qquad \alpha_4 = -.67708$$
$$\alpha_2 = -6.74085 \qquad \alpha_5 = -.93891$$

(iii) It was not necessary to calculate the individual deviations of yield and pre-seasonal precipitation from the regression formulas. The required sums of squares and products of the residuals may be deduced from the sums prior to fitting the regressions by means of the identities (VI) and (VIa) of (8). In the present notation,

$$S(p - \beta_0\rho_0 - \beta_1\rho_1 - \ldots\ldots - \beta_5\rho_5)^2 = S(p^2) - \beta_0 S(p\rho_0) - \beta_1 S(p\rho_1) - \ldots\ldots - \beta_5 S(p\rho_5) \quad \text{(II)}$$

$$S(p - \beta_0\rho_0 - \beta_1\rho_1 - \ldots\ldots - \beta_5\rho_5)(y - \alpha_0\rho_0 - \alpha_1\rho_1 - \ldots\ldots - \alpha_5\rho_5) = S(py) - \alpha_0 S(p\rho_0) - \alpha_1 S(p\rho_1) - \ldots\ldots - \alpha_5 S(p\rho_5) \quad \text{(IIa)}$$

the individual quantities p, ρ and y (yield) being understood to be the annual deviations from the respective station averages.

Applying (II), the sum of the squares of the pre-seasonal precipitation residuals was calculated to be 357.9626. In the same way, 196.6834 was obtained for the sum of the products of the yield and pre-seasonal precipitation residuals by means of (IIa). These gave $\gamma = 0.55$ bushels per acre as the average increase in yield associated with each additional inch of pre-seasonal precipitation. The sum of the squares of the yield residuals (8, Table V) was 6370.15. Table II shows the proportion of this total accounted for by the regression on pre-seasonal precipitation; the mean square due to the regression is actually slightly smaller than the mean square deviation, indicating that the regression coefficient γ is statistically insignificant. Accordingly, it cannot be concluded that there was any consistent relation between pre-seasonal precipitation and yield in this series of data.

TABLE II

ANALYSIS OF RESIDUAL VARIANCE OF YIELD OF MARQUIS WHEAT FROM TEST PLOTS

Variance due to	Degrees of freedom	Sum of squares	Mean square
Regression on pre-seasonal precipitation	1	108.07	108.07
Deviations from regression	56	6262.08	111.82
Total residual	57	6370.15	—

Pre-seasonal Precipitation and Differential Yield of Wheat Varieties

Annual varietal test yields of the four wheat varieties previously mentioned, at each of seven stations for the period 1925–31, have already been tabulated (8, Table II).

(i) The sum of the squares of the seasonal variations in pre-seasonal precipitation from the various station averages for this period was 349.2600. Summing the products of these variations and those of the four varietal yield differences previously studied gave the following values: Garnet-Reward, 125.310; Garnet-Marquis, −13.546; Garnet-Red Fife, 144.283; Marquis-Red Fife 187.070.

(ii) The Normal Equations determining the regression coefficients of pre-seasonal precipitation on the rainfall distribution coefficients for the growing season were:

$$\begin{aligned}
12.441809\beta_0 + .447835\beta_1 - 4.232000\beta_2 + 1.855544\beta_3 \\
+ 2.699285\beta_4 + 5.111341\beta_5 = 17.35489 \\
.447835\beta_0 + 9.186527\beta_1 - .422194\beta_2 - 3.748035\beta_3 \\
+ .565771\beta_4 + .077764\beta_5 = -5.28687 \\
4.232000\beta_0 - .422194\beta_1 + 10.456264\beta_2 + .260742\beta_3 \\
- 4.267647\beta_4 - 3.509379\beta_5 = -5.95805 \\
1.855544\beta_0 - 3.748035\beta_1 + .260742\beta_2 + 7.905838\beta_3 \\
- .955070\beta_4 - 1.870688\beta_5 = 6.72658 \\
2.699285\beta_0 + .565771\beta_1 - 4.267647\beta_2 - .955070\beta_3 \\
+ 16.362681\beta_4 + 1.468324\beta_5 = 8.62844 \\
5.111341\beta_0 + .077764\beta_1 - 3.509379\beta_2 - 1.870688\beta_3 \\
+ 1.468324\beta_4 + 10.407955\beta_5 = 3.86242 \quad \text{(III)}
\end{aligned}$$

whence, by means of the determinantal ratios previously used (8, Table VI)

$$\begin{aligned}
\beta_0 &= 2.67315 & \beta_3 &= -.73721 \\
\beta_1 &= -1.35145 & \beta_4 &= .31405 \\
\beta_2 &= .02076 & \beta_5 &= -.21058
\end{aligned}$$

The previously deduced (8, page 315) regression coefficients α of the varietal yield differences on $\rho_0, \rho_1 \ldots \rho_5$ are collected together in Table III.

TABLE III

REGRESSION COEFFICIENTS OF VARIETAL YIELD DIFFERENCES ON RAINFALL DISTRIBUTION COEFFICIENTS

Regression coefficient	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
α_0	2.23029	.34679	2.98575	1.40547
α_1	−1.21200	1.63877	− .31845	−1.56621
α_2	1.93438	−1.07564	− .45215	−2.69321
α_3	− .69475	2.61538	− .79706	−2.19339
α_4	.07248	2.33236	4.25054	.92497
α_5	− .44483	.39608	.64787	1.08334

(iii) Applying (II), page 000, the sum of the squares of the pre-seasonal precipitation residuals was calculated to be 245.8330. The sums of products of the yield difference and pre-seasonal precipitation residuals, obtained by means of (IIa) were: Garnet-Reward, 77.222; Garnet-Marquis, −93.295; Garnet-Red Fife, −41.043; Marquis-Red Fife, 83.844. These gave partial regression coefficients of yield difference on pre-seasonal precipitation of 0.31, −0.38, −0.17 and 0.34 bushels per acre, respectively, per additional inch.

Table IV shows the extent to which the variance of the yield difference residuals is accounted for by the regression. The degree of correlation indicated is in all cases insignificant.

TABLE IV

ANALYSIS OF RESIDUAL VARIANCE OF VARIETAL YIELD DIFFERENCES

Variance	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
Sum of squares:				
Due to regression on pre-seasonal precipitation (1 deg. freedom)	24.26	35.41	6.85	28.60
Deviations from regression (35 degrees of freedom)	493.92	1686.38	2097.20	856.34
Total residual (36 degrees of freedom)*	518.18	1721.79	2104.05	884.94
Mean square:				
Due to regression on pre-seasonal precipitation	24.26	35.41	6.85	28.60
Deviation from regression	14.11	48.18	59.92	24.47

* *From (8), Table VII.*

Pre-seasonal Precipitation and Yield of Wheat from Summerfallowed and Stubble Land

Wheat yields secured from year to year at the stations maintaining this three-course experimental rotation have already been listed (8, Table III).

TABLE V

REGRESSION COEFFICIENTS OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND ON RAINFALL DISTRIBUTION COEFFICIENTS

Regression coefficient	Summerfallowed land	Stubble land	Difference, summerfallow −stubble
α_0	9.80077	9.09916	.70161
α_1	− .48082	−2.34024	1.85942
α_2	−3.87536	−2.37545	−1.49991
α_3	.00891	.83645	− .82754
α_4	− .70476	−1.61300	.90824
α_5	.19378	.03891	.15488

(i) The required sum of the squares of annual fluctuations in pre-seasonal precipitation was found to be 354.5470. Products of these variations and those of the yield from summerfallowed land, of the yield from stubble land and of the difference in yield from summerfallow and stubble totalled 764.690, 653.652 and 111.038 respectively.

(ii) The previously determined (8) regression coefficients α of yield on the rainfall distribution coefficients ρ for the growing season are given in Table V. The Normal Equations specifying the regression coefficients β of pre-seasonal precipitation on the rainfall distribution coefficients were:

$$\begin{aligned}
12.843475\beta_0 - .335858\beta_1 - 4.365599\beta_2 + 1.826327\beta_3 \\
+ 1.794788\beta_4 + 4.455203\beta_5 = 33.15616 \\
- .335858\beta_0 + 8.950589\beta_1 - .005577\beta_2 - 4.254751\beta_3 \\
+ 1.254894\beta_4 + .280159\beta_5 = - 9.52226 \\
-4.365599\beta_0 - .005577\beta_1 + 9.856559\beta_2 + 2.416812\beta_3 \\
- 4.985248\beta_4 - .724501\beta_5 = -15.06180 \\
1.826327\beta_0 - 4.254751\beta_1 + 2.416812\beta_2 + 10.123139\beta_3 \\
- .217015\beta_4 - .367921\beta_5 = 3.12835 \\
1.794788\beta_0 + 1.254894\beta_1 - 4.985248\beta_2 - .217015\beta_3 \\
+ 15.041716\beta_4 + 1.515575\beta_5 = 7.77009 \\
4.455203\beta_0 + .280159\beta_1 - .724501\beta_2 - .367921\beta_3 \\
+ 1.515575\beta_4 + 10.644241\beta_5 = 10.05162 \quad \text{(IV)}
\end{aligned}$$

From these

$$\begin{aligned}
\beta_0 &= 2.67315 & \beta_3 &= -.73721 \\
\beta_1 &= -1.35145 & \beta_4 &= .31405 \\
\beta_2 &= .02076 & \beta_5 &= -.21058
\end{aligned}$$

(iii) Proceeding as before, the sum of the squares of the pre-seasonal precipitation residuals was found to be 255.3420. The residual sums of products of pre-seasonal precipitation and yield were: summerfallowed land, 380.286; stubble land, 303.421; difference between summerfallowed and stubble land, 76.865. The partial regression coefficients γ of yield on pre-seasonal precipitation were therefore; summerfallowed land, 1.49; stubble land, 1.19; and difference between summerfallow and stubble, 0.30 bushels per acre for each additional inch of pre-seasonal precipitation. Analyses of variance, shown in Table VI, indicated that there was a significant degree of association between pre-seasonal precipitation and the yield from either summerfallowed or stubble

TABLE VI

ANALYSIS OF RESIDUAL VARIANCE OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND

Variance	Summer-fallow	Stubble	Summer-fallow −stubble
Sum of squares:			
Due to regression on pre-seasonal precipitation (1 degree of freedom)	566.37	360.55	23.14
Deviations from regression (35 degrees of freedom)	1931.92	1970.72	1543.51
Total residual (36 degrees of freedom)*	2498.29	2331.27	1566.65
Mean square:			
Due to regression on pre-seasonal precipitation	566.37	360.55	23.14
Deviation from regression	55.20	56.31	44.10

**From (8), Table XI.*

land. Annual variations in the difference in yield between the fallowed and stubble areas, however, evidently were not related consistently to this meteorological factor.

B. Pre-seasonal Rainfall Only

Studies at the Swift Current experimental station extending over a period of seven years are reported by Barnes and Hopkins (1) to have indicated no appreciable increase in soil moisture from the presence of snow cover. This they attribute to the fact that moisture can enter the soil only if the soil temperature is above freezing-point. Such a condition may sometimes prevail in the autumn after the first snowfall, but usually frost follows quickly and the soil becomes frozen before absorbed moisture, derived from melted snow, can penetrate to any appreciable depth.

In these circumstances it might be thought that variations in the amount of winter snowfall from year to year would have little effect on subsequent crop yields, and might in fact merely obscure any real correlation between yield and autumn and spring rainfall. Although this seemed not to be so in the case of the qualitative character, protein content, previously investigated (9), it was nevertheless thought desirable to determine whether the three series of crop yields studied in the preceding section were more closely correlated with pre-seasonal rainfall alone than with the total of pre-seasonal rain and snow. Accordingly, the annual quantities of pre-seasonal precipitation given in Table I were separated into rain and snow, and the amounts of rain entered in Table VII.

TABLE VII

Pre-seasonal precipitation (rain only) in inches

Crop year	Edmonton	Lacombe	Lethbridge	Indian Head	Swift Current	Scott	Rosthern	Saskatoon
1931	3.07	5.42	3.72	2.32	4.73	3.17	3.80	3.36
1930	3.27	3.06	3.83	2.87	2.11	2.44	2.20	1.80
1929	3.63	3.71	2.33	1.10	1.34	0.93	1.95	2.41
1928	3.22	4.95	5.61	6.19	5.83	4.41	5.12	4.25
1927	7.50	10.23	8.35	5.55	4.76	5.05	3.68	3.32
1926	4.53	9.67	7.92	4.00	2.65	3.92	6.70	5.03
1925	4.17	5.98	5.03	5.69	6.31	5.21	7.10	4.67
1922	2.36	2.68	3.66	8.98	—	2.32	4.13	5.58
1921	4.06	4.95	2.16	6.53	—	5.81	6.09	5.84
1920	3.33	5.56	4.14	3.56	—	4.24	5.22	4.72

In estimating the partial regression of each series of wheat yields on pre-seasonal rainfall, the procedure employed in the preceding section was again followed, with the substitution of the rainfall indicated in Table VII for the corresponding amount of total precipitation shown in Table I.

Pre-seasonal Rainfall and Yield of Marquis Wheat

(i) Seasonal covariation of yield and pre-seasonal rainfall gave rise to a sum of products of 419.993. The sum of the squares of fluctuations in rainfall amounted to 229.4541.

(ii) The left-hand side of the Normal Equations to determine the regression coefficients β_0', β_1', ... β_5' of pre-seasonal rainfall p' on the summer rainfall coefficients ρ_0, ρ_1, ... ρ_5 remained as in (I). It was necessary however to replace the numerical quantities on the right-hand side by the sums of products generated by the seasonal covariation of p' and ρ_0, ρ_1, ... ρ_5, namely 26.57925, −6.18413, −7.89021, 9.28615, 3.57478 and 1.69060. Making this substitution, the regression coefficients β' were found to be:

$\beta_0' = 1.65587$ $\quad$ $\beta_3' = .43480$
$\beta_1' = -.36322$ $\quad$ $\beta_4' = -.08300$
$\beta_2' = .10801$ $\quad$ $\beta_5' = -.01235$

(iii) These calculations provided the data necessary for the adjustment of the crude sums of squares and products of yield and pre-seasonal rainfall by means of (II) and (IIa) to give the corresponding functions of the residuals. The residual pre-seasonal rainfall sum of squares was 180.3284, and the residual sum of products of yield and pre-seasonal rainfall 203.541. The partial regression coefficient γ' of yield on pre-seasonal rainfall was therefore 1.13 bushels per acre for each additional inch of total pre-seasonal precipitation.

Table VIII shows the distribution of the residual yield variance after fitting the regression. The mean square due to the regression now exceeds the

TABLE VIII

ANALYSIS OF RESIDUAL VARIANCE OF YIELD OF MARQUIS WHEAT FROM TEST PLOTS

Variance due to	Degrees of freedom	Sum of squares	Mean square
Regression on pre-seasonal rainfall	1	229.74	229.74
Deviations from regression	56	6140.41	109.65
Total residual	57	6370.15	—

mean square deviation, but the ratio of these corresponds to a z value (7, Sec. 47) of only 0.370, whereas the 5% point for 1 and 56 degrees of freedom is approximately 0.700. It must be concluded therefore, that although there was some association between pre-seasonal rainfall and yield in the sample of seasons examined, it is not improbable that this was an entirely chance effect.

Pre-seasonal Rainfall and Differential Yield of Wheat Varieties

(i) For this series, the sum of the squares of the annual variations in pre-seasonal rainfall was 174.4978. The sums of the products of the variations in pre-seasonal precipitation and in the four yield-differences were: Garnet-Reward, 53.451; Garnet-Marquis, −46.686; Garnet-Red Fife, 13.205; Marquis-Red Fife, 90.234.

(ii) The sums of products of p' and ρ_0, ρ_1, ... ρ_5, to be substituted in the right-hand side of Normal Equations (III) were 17.35065, −6.18374,

−3.33279, 4.83174, 1.77350, and 3.69845. These resulted in the subjoined values of β':

$$\beta_0' = 1.75868 \qquad \beta_3' = -.35544$$
$$\beta_1' = -.88800 \qquad \beta_4' = -.08418$$
$$\beta_2' = .16442 \qquad \beta_5' = -.49808$$

(iii) Employing these coefficients in (II) and (IIa) as before gave 142.7492 for the sum of the squares of the residual deviations of pre-seasonal rainfall, and for the residual sums of products of pre-seasonal rainfall and the yield differences: Garnet-Reward, 18.580; Garnet-Marquis, −64.392; Garnet-Red Fife, −48.159; Marquis-Red Fife 52.138. The partial regression coefficients γ' of the yield-differences on pre-seasonal rainfall were therefore 0.13, −0.45, −0.34 and 0.37 bushels per acre per inch respectively. As is shown by the

TABLE IX

ANALYSIS OF RESIDUAL VARIANCE OF VARIETAL YIELD DIFFERENCES

Variance	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
Sum of squares:				
Due to regression on pre-seasonal rainfall (1 degree of freedom)	2.42	29.05	16.25	19.04
Deviations from regression (35 degrees of freedom)	515.76	1692.74	2087.80	865.90
Total residual (36 degrees of freedom)	518.18	1721.79	2104.05	884.94
Mean square:				
Due to regression on pre-seasonal rainfall	2.42	29.05	16.25	19.04
Deviation from regression	14.74	48.36	59.65	24.74

analysis of variance in Table IX, these are all statistically insignificant, and provide no evidence of any consistent effect of variations in the amount of pre-seasonal rain on the relative yield of the four varieties of wheat.

Pre-seasonal Rainfall and Yield of Wheat from Summerfallowed and Stubble Land

(i) In this case, the sum of the squares of the annual deviations from the station averages of pre-seasonal rainfall was 193.5744. The corresponding sums of products of yield and pre-seasonal rainfall were: summerfallowed land, 488.182; stubble land, 452.653; difference, summerfallow−stubble, 35.529.

(ii) Summing the products of p' and ρ_0, ρ_1, ... ρ_5 gave 21.14056, −2.90190, −2.57160, 4.67500, −3.01081 and 1.27903. Substituting these in Equations (IV), the following regression coefficients β^1 were found:

$$\beta_0' = 2.15063 \qquad \beta_3' = -.23254$$
$$\beta_1' = -.30911 \qquad \beta_4' = -.15812$$
$$\beta_2' = .61592 \qquad \beta_5' = -.71546$$

(iii) The residual pre-seasonal rainfall sum of squares, deduced as before, was 150.3218, and the residual sums of products of yield and pre-seasonal rainfall were 267.216, 238.575 and 28.641. These yielded the following partial regression coefficients γ': summerfallowed land, 1.78; stubble land 1.59; and difference summerfallow-stubble 0.19 bushels per acre for each additional inch of pre-seasonal rain.

It will be seen by reference to Table X that the variance of yield from both the fallowed and stubble land, accounted for by the regression, significantly exceeded the mean square deviation, the z values being 1.053 and 0.958 respectively (5% point = 0.707). No significant differential effect of pre-seasonal rain on the yield from summerfallow and stubble was demonstrable.

TABLE X

ANALYSIS OF RESIDUAL VARIANCE OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND

Variance	Summer-fallow	Stubble	Summer-fallow —stubble
Sum of squares:			
Due to regression on pre-seasonal rainfall (1 degree of freedom)	475.01	378.64	5.46
Deviation from regression (35 degrees of freedom)	2023.28	1952.63	1561.19
Total residual (36 degrees of freedom)	2498.29	2331.27	1566.65
Mean square:			
Due to regression on pre-seasonal rainfall	475.01	378.64	5.46
Deviation from regression	57.81	55.79	44.61

The regression coefficients γ' of yield on pre-seasonal rainfall are slightly larger than the coefficients γ previously obtained with the total of pre-seasonal rain and snow as the independent variable, suggesting that precipitation in the form of rain was more efficient in augmenting yield than was a corresponding amount of snow. On the other hand, they accounted for a somewhat lower proportion of the yield variance, indicating that the correlation between pre-seasonal precipitation and yield was higher when the total of both rain and snow was considered. The difference is not, however, statistically significant, having regard to the number of observations. There may have been some additional effect of snow, but on the whole it seems probable that the major part of any increase in yield resulting from pre-seasonal precipitation was attributable to autumn or spring rainfall.

Significant effects of this nature may be of considerable agricultural importance. As previously intimated, soil conditions in the plots subjected to this experimental grain rotation probably approximated fairly closely to those met with in general farming practice. The varietal test plots on the other hand constituted a special case, and a comparison of the two sets of meteorological correlations throughout the investigation is indicative of the manner in which cultural practices may modify the incidence of weather effects.

III. Relation Between Precipitation and Agricultural Yields

As was pointed out elsewhere (8, Section 1), any detailed analysis of the relation between weather conditions and agricultural yields would require a series of records more extensive than any that have so far come to the author's attention. However, the results of the study of plot yields had indicated that the amounts of precipitation prior to and during the growing season were likely to be the most important individual weather factors, although the possibility of significant non-linear temperature effects should not be overlooked (2, 10). It was felt, therefore, that a brief examination of the correlation of the former with some available agricultural yield data might provide some indication of the degree of accuracy likely to be attained by a simple yield prediction equation.

For the purpose of crop reporting, the prairie provinces have each been divided into a number of crop districts. The annually reported yields of wheat in six of these districts, three in the central and three in the southern regions of Saskatchewan and Alberta, were selected for study. These districts centered on Lacombe, Scott and Saskatoon, and on Calgary, Swift Current and Regina respectively. Records of the average annual wheat yield by crop districts were available as far back as the year 1908, but the boundaries of the districts as originally constituted later underwent considerable modification. For this reason, it was judged that only the data from 1916 onwards were sufficiently homogeneous for correlational purposes.

The average wheat yield for each district is computed by the provincial Departments of Agriculture from estimates supplied by farmers in the various townships included, supplemented by threshermen's returns. Consequently, the figures given may be affected to some extent by subjective bias. It is probable however that the limitations of the meteorological data with which these were correlated introduced a greater source of error, since for four of the six districts continuous records at a single station only were available. The magnitude of local variations in the incidence of rainfall is now under investigation. In the meantime it must be suspected that observations at one or two points did not represent accurately the average conditions prevailing in different years over the areas in question.

It has been shown by Fisher (6) that the distribution of the multiple correlation coefficient R, in samples from populations of actually zero association, has a positive bias specified by the ratio of the number of independent variables involved to the number of residual degrees of freedom. High correlations between several variables, deduced from short series of observations, may be expected therefore to be the outcome in part at least of chance, rather than truly causal, association, and to require modification in the light of subsequent experience. It is not clear that this circumstance was taken into consideration by Bogue (3) who has recently reported high correlations between weather and wheat yield in the prairie provinces generally.

As the crop yields now under consideration comprised only 19 years' records it was clearly undesirable in view of the foregoing considerations, rein-

forced as they were by the doubtful adequacy of the meteorological data, to attempt any detailed correlation of yield and seasonal precipitation. The latter was therefore specified merely by the total amounts received during the preceding period from August 1 to April 30 inclusive, and during the period of active crop growth (May 1 to July 31) each year.

The regression of yield on pre-seasonal and summer precipitation, thus defined, was first determined for each of the six districts individually. It was found that there was in all cases a significant correlation between precipitation and the yield recorded, that there were no statistically significant differences between the results for the three central districts or between those for the three southern ones, but that the residual deviations were relatively large. Accordingly, the correlation between yield and precipitation in the annual averages of the three central and the three southern districts, which would be somewhat less affected by random inaccuracies in the data, was next examined.

Both series of yield and precipitation averages are given in Table XI. In arriving at the total pre-seasonal precipitation, the conventional con-

TABLE XI

AVERAGE WHEAT YIELD (BU. PER ACRE) AND PRECIPITATION (INCHES OF RAIN) IN CENTRAL AND SOUTHERN DISTRICTS

Year	Central Districts			Southern Districts		
	Yield	Precipitation		Yield	Precipitation	
		Preceding, Aug. 1–Apr. 30	May 1–July 31		Preceding, Aug. 1–Apr. 30	May 1–July 31
1916	18.0	6.08	9.61	16.3	8.33	8.34
1917	15.2	9.54	3.82	16.9	8.02	3.53
1918	11.8	7.29	4.44	10.3	7.09	3.67
1919	15.5	6.94	4.38	9.6	7.69	3.34
1920	15.4	9.97	5.46	18.6	8.65	7.78
1921	14.2	9.72	6.95	13.5	7.05	7.29
1922	13.8	6.23	4.13	20.4	8.64	6.91
1923	25.6	7.03	11.28	23.7	7.10	12.85
1924	8.1	7.67	3.66	16.0	7.38	6.24
1925	19.7	11.45	6.49	20.0	11.76	6.34
1926	15.8	9.98	6.74	19.5	9.22	7.27
1927	21.5	11.09	9.64	19.5	12.68	11.26
1928	22.8	8.63	8.51	24.7	11.28	8.80
1929	11.2	5.31	4.30	8.1	5.25	4.97
1930	16.6	5.74	5.40	12.7	8.00	4.80
1931	15.2	6.08	7.58	7.4	5.56	5.11
1932	16.2	9.70	6.76	14.4	9.78	8.49
1933	8.7	7.76	5.05	11.0	8.01	6.66
1934	10.2	7.72	6.63	7.4	9.45	5.75

version factor, 0.1, was used to estimate the rainfall equivalent of snow. It may be noted that annual variations in the average yield recorded were more pronounced in the southern than in the central districts.

Denoting by b_1 and b_2 the regression coefficients of yield on precipitation (x_1) during the period, May 1 to July 31 of the crop year, and on that (x_2)

during the period from August 1 of the preceding year to April 30 of the crop year respectively, the Normal Equations determining these were computed from the data for the central districts, shown in Table XI, to be:

$$86.3931\ b_1 + 11.5969\ b_2 = 140.005$$
$$11.5969\ b_1 + 63.3356\ b_2 = 42.670$$

giving $b_1 = 1.57 \pm 0.33$ and $b_2 = 0.39 \pm 0.39$ bushels per acre for each additional inch of precipitation.

For the southern district averages, the Normal Equations found were:

$$111.4669\ b_1 + 36.3307\ b_2 = 161.475$$
$$36.3307\ b_1 + 66.8299\ b_2 = 105.393$$

giving $b_1 = 1.14 \pm 0.40$ and $b_2 = 0.96 \pm 0.52$ bushels per acre for each additional inch of precipitation.

In both cases the regression on precipitation during the period May 1 to July 31 was significant, and of the same order, the difference between the two values of b_1 being statistically insignificant. The yields reported for the southern districts apparently were affected also by the amount of pre-seasonal precipitation, the coefficient b_2 being 1.85 times its standard error. This ratio would be expected to be attained on the average only about 8 times in 100 as a chance result. For the central districts, on the other hand, the coefficient b_2 does not exceed its standard error.

This more definite indication of dependence on pre-seasonal precipitation in the southern districts perhaps resulted in part from greater losses of soil moisture by evaporation during the late summer and autumn months. Thus for the six-year period 1925–30, the average amounts of evaporation from a free-water surface during August and September at Lacombe (central Alberta) and Swift Current (southern Saskatchewan) were calculated from annual data published in (4) and (5) to be:

	August	September
Lacombe	3.53 in.	2.14 in.
Swift Current	6.43 in.	3.82 in.

It might be supposed that rainfall during the summer months (May to July) of the year previous to cropping exerts a sensible influence on yield, through mosture conserved in the summerfallowed acreage; but this could not be established from the present data. Including this as a third independent variable, x_3, the Normal Equations determining the regression coefficients became:

$$86.3931\ b_1 + 11.5969\ b_2 - 13.7365\ b_3 = 140.005$$
$$11.5969\ b_1 + 63.3356\ b_2 - 3.6792\ b_3 = 42.670$$
$$-13.7365\ b_1 - 3.6792\ b_2 + 89.7921\ b_3 = -36.701$$

and

$$111.4669\ b_1 + 36.3307\ b_2 + 23.9322\ b_3 = 161.475$$
$$36.3307\ b_1 + 66.8299\ b_2 + 6.8354\ b_3 = 105.393$$
$$23.9322\ b_1 + 6.8354\ b_2 + 111.7377\ b_3 = 73.387$$

which gave $b_1 = 1.54$, $b_2 = 0.38$, $b_3 \doteq -0.16$ bushels per acre for the central district averages and $b_1 = 1.05$, $b_2 = 0.97$, $b_3 = 0.37$ bushels per acre for the southern district averages for each additional inch of rain during the periods specified. In both cases the regression of yield on the previous summer's rain was insignificant, as is shown by the analyses of variance in Table XII.

TABLE XII

ANALYSIS OF VARIANCE OF AVERAGE WHEAT YIELD IN CENTRAL AND SOUTHERN DISTRICTS

Variance	Degrees of freedom	Central Districts		Southern Districts	
		Sum of squares	Mean square	Sum of squares	Mean square
Accounted for by regression on precipitation during May 1–July 31, and preceding Aug. 1–Apr. 30	2	236.11	118.06	284.55	142.28
Additional effect of precipitation during May 1–July 31 of previous year	1	2.16	2.16	14.74	14.74
Residual	15	144.70	9.65	221.37	14.76
Total	18	382.97	—	520.66	—

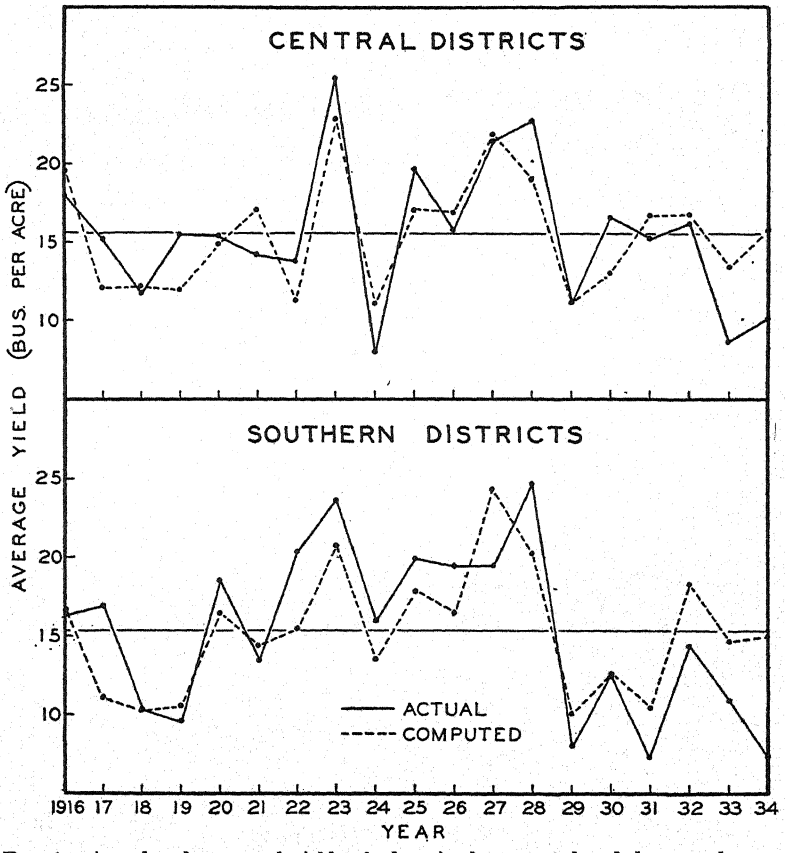

FIG. 1. *Actual and computed yields of wheat in three central and three southern crop districts. Horizontal lines indicate average yield, 1916–34.*

Reverting to the previously determined regression of yield on x_1 and x_2 only, the coefficient of multiple correlation R between average precipitation and yield was 0.74 for the central districts and 0.79 for the southern ones. Fig. 1 illustrates the agreement between the average yields actually reported and those calculated (Y) from the regression equations

$$Y = 2.41 + 1.57x_1 + 0.39x_2$$
$$Y = -0.63 + 1.14x_1 + 0.96x_2$$

for the central and southern districts respectively.

If this degree of reliability could be maintained in the estimation of future crops, prediction equations even of this simple nature might be of some use particularly in view of the early date (end of July) at which estimates could be made. Nevertheless, the accuracy attained still leaves a good deal to be desired. The standard deviation of the actual from the calculated yields, as computed from the mean square residual (16 degrees of freedom) was 3.0 bushels per acre for the central and 3.8 for the southern district averages, and as the area annually devoted to wheat growing in the prairie provinces is of the order of 20,000,000 acres, the total production estimated in this way will undoubtedly be greatly in error in some seasons. It remains to be seen whether future refinements, such as the accumulation of meteorological records at an increased number of stations in each district, the determination of the limits of effective rainfall, and the incorporation of additional weather variables (when the series of annual yields has become sufficiently numerous) will bring this uncertainty within the limits of practical application.

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THE ABSORPTION OF NUTRIENTS BY TWO VARIETIES OF WHEAT GROWN ON THE BLACK AND GRAY SOILS OF ALBERTA[1]

BY E. K. WOODFORD[2] AND A. G. MCCALLA[3]

Abstract

Chemical analyses carried out at five stages of development of Reward and Red Bobs wheat grown on the black and the gray soils of Alberta showed that differences in soil and variety significantly influenced the composition of the plants.

The weights of dry matter and all nutrients studied were higher for the black-soil plants.

On the basis of percentage dry matter all nutrients, except phosphorus, were higher in the black-soil plants. Reward was higher than Red Bobs in nitrogen when grown on the black soil; and in ash, phosphorus and potassium when grown on the gray soil.

The grain of gray-soil plants was higher in all ash constituents but lower in nitrogen. Varietal differences were more marked in the grain and straw of the mature plants, Reward grain grown on both soils being higher in nitrogen, ash, phosphorus and magnesium.

The total weights, percentages, rates of absorption and ion ratios all indicated that nitrogen and sulphur were limiting the growth of wheat on the gray soil. It is suggested that the proportionately higher absorption of phosphorus from the soil was in compensation for the low availability of nitrogen and sulphur.

The differences in original quality of the wheats grown on the two soils can be largely accounted for by the differences in protein content, and therefore nitrogen supply. Phosphorus absorption, nitrogen, phosphorus and sulphur balance, and the relation of ash to protein, are possibly important in determining the keeping properties of the flour.

Introduction

The black and gray soils of Alberta differ markedly in their chemical composition (23) and in their ability to produce crops of high yield and quality. Larger yields of both grain and forage crops are always obtained on the black soil, and the differences in composition of the crops grown on the two soils cannot be entirely explained by the known differences in soil composition. The quality of the wheat grown on the gray soil is poorer than that of the same varieties grown on the black soil, and the flours milled from most of the varieties deteriorate more rapidly on storage (1). Flour milled from black-soil wheat can be stored for two years without marked deterioration, while flour from similar wheat grown on the gray soil is unfit for commercial bread-making purposes after one year's storage.

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A thorough understanding of the amounts and distribution of the principal nutrients in the wheat plant appeared to be an essential preliminary to the study of the detailed physiological processes which might be responsible for the differences in quality and keeping properties of the flour milled from different varieties of wheat grown on the two soils. The progressive changes in the chemical composition of the developing wheat plant have been followed by many workers, and results from various parts of the world are available. The work of Knowles and Watkin (7) in England is the most comprehensive of recent years. The primary object of these and similar experiments has been, however, to follow the growth of the wheat plant and not to study changes in composition that are caused by soil and varietal differences. Recent work reported from French experimental stations (3, 11, 12, 16) on the chemical composition of different varieties grown on different soils has indicated the magnitude of some of the chemical differences that may be expected. These studies have unfortunately been made at only one or two stages in the plant's development and, as far as the writers are aware, there is no comprehensive work covering this question over the whole life period of the wheat plant. No detailed work has been attempted to relate the chemical development of the wheat plant to the quality and keeping properties of the wheat and flour. The present paper presents the result of such a study in which the absorption of nutrients by two varieties of wheat grown on the black and gray soils was followed over the whole period of development of the plants.

Experimental Material

The material used in this study was grown on fallowed land during the spring and summer of 1934. The experimental plots maintained at Edmonton are typical of the black soil, and those at Fallis of the gray soil areas. Edmonton and Fallis are 50 miles apart, but are characterized by similar climatic conditions. The rainfall is probably slightly higher at Fallis but no meteorological data are available. Since the growing of legumes on the gray soil has been shown to affect the yield and composition of subsequent crops, soil which had never grown legumes was selected for this experiment.

The composition of the two soil types is described by Wyatt and Newton (23) who state that the greatest differences occur in the first foot, where the black soil contains about four times as much nitrogen and more than twice as much phosphorus as the gray soil. There are likewise greater quantities of nitrogen, phosphorus, calcium and magnesium in the second and third foot depths of the black soil than in the corresponding depths of the gray soil.

Two varieties of wheat, Reward and Red Bobs, were grown. Reward was chosen because when grown on the gray soil it had the highest quality of all varieties previously tested, and in addition was the only standard variety commonly grown in this area which produced flour that did not deteriorate rapidly on storage. Red Bobs, when grown on the gray soil, had a marked tendency to produce starchy kernels, and yielded a flour with somewhat poorer original quality and decidedly poorer keeping properties. These two

varieties are, according to grading standards, equal in quality to Marquis and are admitted to all the statutory hard red spring wheat grades.

The experiment was planned to facilitate the statistical analysis of the data. Each variety was replicated eight times on each soil. The varieties were grown side by side in blocks 18 feet long and 12 rows wide. The rows were one foot apart and each variety occupied a sub-block six rows wide. The position of the variety in each block was determined at random. Blocks were adjacent to each other and the whole plot was surrounded by three guard rows.

The plots were sampled five times. Each sample from a sub-block represented the entire length of the block except for a nine inch section at each end which was discarded to remove border effect. The collections were made by a "staircase" method of sampling (Fig. 1). A six inch sample was taken from each row and 27 inches were left between the samples in adjacent rows. The rows in each sub-block and each six inch section in a row were numbered. The starting row and the position of the sample in that row were randomized by the use of Tippet's (18) random sampling numbers. Randomization for all the collections was made at the same time and, if overlapping occurred, the starting point in the row was moved so that two samples were never taken from adjacent six inch sections.

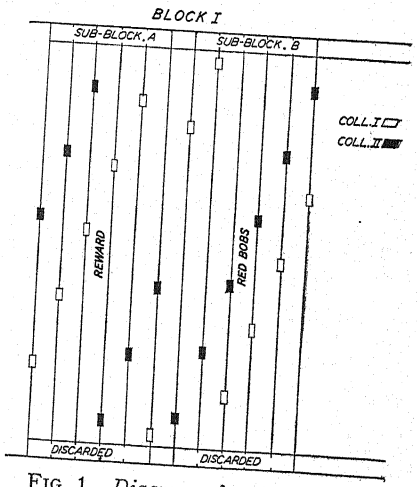

FIG. 1. *Diagram of a section of plot and the method of sampling for the first and second collections.*

Edmonton plants were cut at the ground level and immediately transferred to the laboratory for chemical analyses. Fallis plants were dug from the soil and all the plants from a sub-block transferred to an individual container. In this manner the plants, with roots well covered with soil, were prevented from drying out while they were transported to the laboratory where the tops were cut off just above the ground level immediately before analysis.

Comparable collections were made when the plants were at approximately the same stage of development. Five samplings were made as indicated in Table I. The date of each of the first two collections was governed only by the time from seeding. Collection 3 was made when

TABLE I

Collection	Date of collection		Days from seeding	
	Edmonton	Fallis	Edmonton	Fallis
1	June 6	June 7		
2	June 26	June 27	29	29
3	July 11	July 12	48	48
4	Aug. 13	Aug. 7	64	64
5	Aug. 31	Aug. 31	97	90
			115	114

the plants were fully headed, Collection 4 when the grain contained approximately 45% moisture, and Collection 5 at maturity. The heads and straw of Collection 4, and grain and straw of Collection 5, were analyzed separately.

Methods of Analysis

The following determinations were made on each of the eight replicates of each variety, grown at Edmonton and Fallis.

Dry matter. The smaller samples of Collections 1 and 2 were dried directly in a 100° C. air oven. The larger samples of the later collections were rapidly dried in a blast oven at 75° C., then ground in a Wiley mill and further dried at 100° C.

Total nitrogen. Total nitrogen in the vegetative parts was determined by the reduced iron method of Pucher *et al.* (15) in order to include any nitrate nitrogen which was present. The Kjeldahl method, with mercuric oxide as a catalyst, was used to determine nitrogen in the grain, since it has been shown that nitrate nitrogen, if present at all, is negligible.

Non-protein nitrogen. Fresh green material, ground as finely as possible in a food chopper, was extracted with 2.5% trichloracetic acid, and the total nitrogen of this extract determined by the reduced iron method. The procedure was essentially that used by McCalla (9).

Ash. Approximately 2-gm. samples of ground straw or 5-gm. samples of grain were ashed in a muffle furnace at 650° C. for six hours. The ash was taken up in hot dilute hydrochloric acid and the solution diluted to 100 cc. Aliquots of this solution were used in the determination of all the mineral elements studied except sulphur.

Phosphorus. Phosphorus was determined by the colorimetric method described by Truog and Meyer (19) and is reported as phosphorus pentoxide.

Sulphur. Total sulphur was determined only on composite samples of each variety at each place. Approximately 1.5 gm. of the straw, or 0.75 gm. of the grain, was fused in a Parr heat ignition bomb with 12 gm. of sodium peroxide and 0.5 gm. of zinc. The fused mass was dissolved in hot water, made slightly acid, and then filtered. The sulphate was precipitated as barium sulphate and weighed directly. The procedure was an adaptation of the method described by Wolkoff (20).

Potassium. Potassium was precipitated with sodium cobaltic nitrite and the precipitate titrated with $N/20$ potassium permanganate. The details of the procedure are described by Hibbard and Stout (6).

Calcium. Calcium was precipitated as the oxalate and titrated with $N/20$ potassium permanganate.

Magnesium. Magnesium in the filtrate from the calcium determination was precipitated as magnesium ammonium phosphate, dissolved in $N/14$ sulphuric acid, and the excess titrated with $N/14$ sodium hydroxide, methyl red being used as an indicator.

Results

The amounts of the individual nutrients absorbed up to the time of each collection are expressed, first, as total weights; second, as percentages of dry matter; and third, as percentages of total ash. The individual results for each sub-block were too numerous for presentation. The total weights and the mean percentages are presented in graphical form and also in tables with accompanying t values which indicate the significance of the differences due to soil and variety.

The test for significance employed throughout is that of "Student" as described by Fisher (4). The experiment provided eight replicate determinations for each variety at each place. These are taken as providing eight replicate determinations of the differences between Reward and Red Bobs at Edmonton and Fallis. The average of the eight individual differences provided an estimate of the mean difference, and their variance the basis of testing;—(a) whether the mean difference at either place differed significantly from zero (*i.e.*, whether there was any real difference between the varieties) and (b) whether the differences between the varieties were the same at Edmonton and Fallis. In a similar manner, by the use of totals instead of differences, it was determined whether the general level of the various constituents was significantly higher at either Edmonton or Fallis.

These three sets of calculations were made for most of the data. In each of the tables concerned the first column of t values indicates the significance of the difference in the general level at Edmonton and Fallis. The value for the Fallis sample was always subtracted from the value for the Edmonton sample, and in consequence a negative sign means that the general level was higher at Fallis. The second and third columns of t values indicate the significance of the differences between varieties at Edmonton and Fallis, a negative sign meaning that the weight or percentage was higher for Red Bobs than for Reward. The fourth column of t values shows the significance of the difference between the varietal reactions, a high t value indicating that the differences between the varieties were not the same at Edmonton as at Fallis.

Total Weights — Composition of the Whole Plant

The total weights of dry matter, nitrogen, ash and ash constituents are shown in Fig. 2. In the graphs each point, except for sulphur, represents the total of eight replicate samples. The numerical values for these results, together with t values indicating the significance of the differences due to soil and variety, are shown in Table II. Sulphur was determined only on a composite sample from each collection; thus it was impossible to use these data for statistical analysis.

The weights of dry matter and all constituents determined were higher in the Edmonton samples; and the magnitude of this difference, except for potassium and sulphur, increased with the age of the plants. Potassium and sulphur were excreted from the vegetative parts of the plants and the greatest differences in weights occurred before excretion had started. At the point of

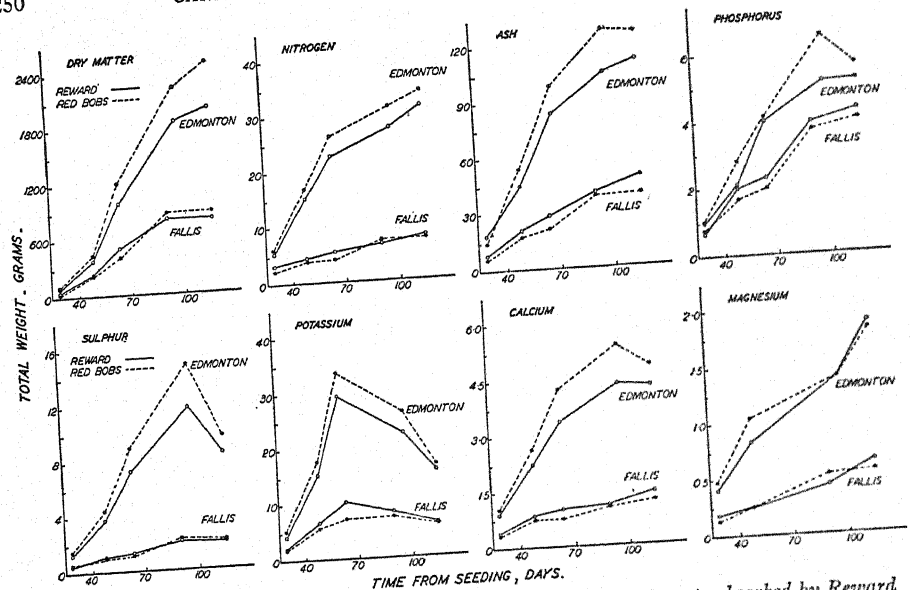

FIG. 2. *Total weights of dry matter, nitrogen, ash and ash constituents absorbed by Reward and Red Bobs from a unit area of soil.*

TABLE II

TOTAL WEIGHTS AND THE SIGNIFICANCE OF DIFFERENCES DUE TO SOIL AND VARIETY

Determination	Collection	Total weight, gm.				*t* values			
		Edmonton		Fallis		Difference between general levels at Edmonton and Fallis	Difference between varieties†		Difference between varietal reactions at Edmonton and Fallis
		Reward	Red Bobs	Reward	Red Bobs		Edmonton	Fallis	
Dry matter	1	100.6	122.5	59.4	53.9	7.11**	−3.12**	0.85	2.81*
	2	380.0	443.4	236.5	233.0	5.57**	−1.60	0.05	1.17
	3	990.4	1223.7	501.2	406.7	25.37**	−4.17**	1.68	4.52**
	4	1863.4	2357.8	814.0	884.3	11.51**	−0.43	0.05	0.34
	5	2011.4	2487.1	827.9	884.6	21.08**	−0.64	−0.46	0.07
Nitrogen	1	5.77	6.74	3.11	2.81	8.21**	−2.30*	0.74	2.16*
	2	15.43	17.07	4.68	4.15	17.35**	−1.13	0.34	1.89
	3	22.98	26.43	5.61	4.27	19.30**	−4.56**	1.68	4.41**
	4	27.53	31.50	6.99	7.54	18.52**	−2.40*	−0.32	1.47
	5	31.04	34.43	8.18	7.79	16.07**	1.76	0.03	1.23
Ash	1	18.81	15.01	8.38	6.90	9.09**	−1.00	1.59	1.83
	2	46.74	54.54	20.81	18.17	12.59**	−2.63*	1.18	2.69*
	3	85.27	99.61	29.47	22.49	13.86**	−5.01**	2.72*	5.47**
	4	106.48	128.63	41.32	40.71	11.50**	1.16	0.95	0.14
	5	112.17	124.04	49.78	40.02	11.32**	−2.28*	0.10	1.69
Phosphorus (P_2O_5)	1	1.02	1.04	0.67	0.72	2.81*	−0.77	−0.46	0.22
	2	2.28	2.71	2.13	1.70	0.93	−1.65	1.21	2.02
	3	3.96	4.16	2.71	2.07	6.53**	−0.54	2.08	1.85
	4	5.19	6.69	3.98	3.71	17.28**	−2.73*	0.44	2.24*
	5	5.31	5.86	4.24	4.04	2.73*	0.94	−0.01	0.67

TABLE II—*Concluded*

TOTAL WEIGHTS AND THE SIGNIFICANCE OF DIFFERENCES DUE TO SOIL AND VARIETY

Determination	Collection	Total weight, gm.				*t* values			
		Edmonton		Fallis		Difference between general levels at Edmonton and Fallis	Difference between varieties†		Difference between varietal reactions at Edmonton and Fallis
		Reward	Red Bobs	Reward	Red Bobs		Edmonton	Fallis	
Potassium	1	4.52	5.23	2.16	2.02	7.12**	−1.29	−0.47	0.58
	2	15.58	17.96	6.86	5.90	11.63**	−1.84	0.75	1.83
	3	29.61	33.90	10.32	7.24	12.24**	−3.23**	1.95	3.66**
	4	22.48	26.34	8.56	7.79	13.43**	−2.03	0.40	1.72
	5	15.80	19.93	6.25	5.70	11.41**	−0.80	0.32	0.79
Calcium	1	0.91	1.09	0.43	0.38	10.57**	−3.21**	0.86	2.88*
	2	2.24	2.66	0.87	0.77	15.92**	−1.88	0.28	1.53
	3	3.37	4.28	1.00	0.77	12.47**	−6.47**	2.04	6.01**
	4	4.37	5.50	1.11	1.10	17.83**	−3.76**	0.00	2.66*
	5	4.30	4.86	1.44	1.24	13.03**	0.85	0.62	0.16
Magnesium	1	0.42	0.48	0.18	0.14	11.30**	−1.62	1.37	2.11
	2	0.84	1.06	0.26	0.26	13.92**	−2.45*	0.28	1.93
	4	1.42	1.42	0.44	0.53	12.45**	0.35	−0.42	0.55
	5	1.84	1.77	0.66	0.56	12.63**	1.92	0.63	0.92

† *A minus sign indicates that the weight for Red Bobs is higher than for Reward.*
* *5% point, t = 2.15.* ** *1% point, t = 2.98.*

the maximum difference the quantities of all constituents except phosphorus, were approximately four times as high in the Edmonton as in the Fallis plants. Phosphorus was exceptional; the differences between Edmonton and Fallis plants being much smaller throughout growth and insignificant in Collection 2.

The differences between varieties indicated that Red Bobs absorbed more nutrients than Reward at Edmonton and less at Fallis, but this difference was significant for less than one-third of the determinations.

Percentage Data

Dry matter as a percentage of green weight. The percentages of dry matter in the fresh samples of the first four collections are shown in the top left hand graph of Fig. 3. The numerical results and *t* values are shown in Table III. Collection 5 is not considered, as it was made after the plants were mature.

Fallis samples were definitely higher in dry matter than corresponding Edmonton samples, and Red Bobs was higher than Reward for both Edmonton and Fallis plants, the latter difference being significant in all but Collections 2 and 3 at Edmonton. Dry matter content is often used as an indication of the stage of maturity of the plant, and composition results are sometimes plotted against the percentage of dry matter instead of the number of days from seeding. In this experiment the percentage of dry matter did suggest the earlier maturity of the Fallis plants, but the higher dry matter content of Red Bobs was not a true indication of maturity since this variety was later than Reward on both soils.

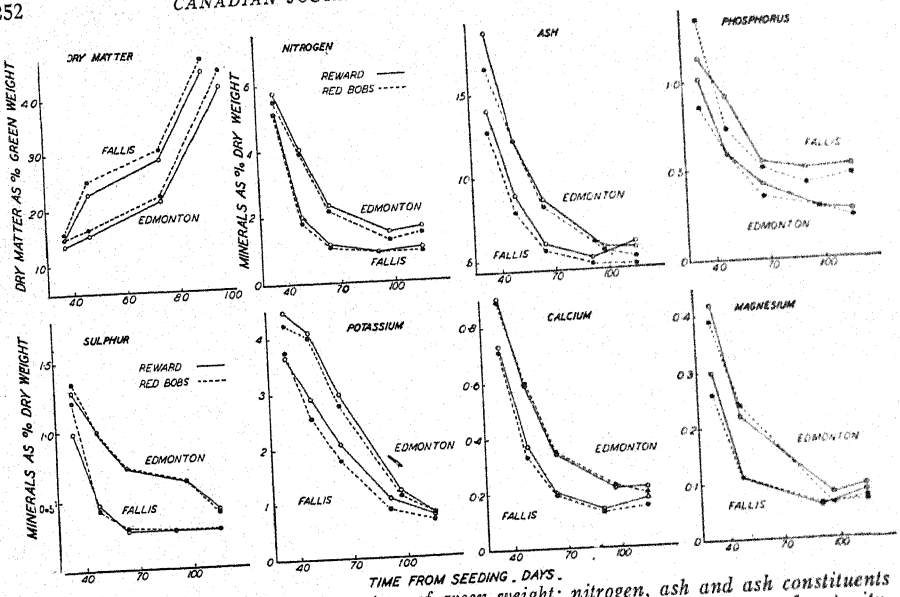

FIG. 3. *Dry matter as a percentage of green weight; nitrogen, ash and ash constituents as a percentage of dry matter in Reward and Red Bobs plants at progressive stages of maturity.*

Nitrogen, ash, and ash constituents, as percentages of dry matter. The average values for nitrogen, ash, and ash constituents expressed as percentages of dry matter are presented graphically in Fig. 3 and numerically, together with *t* values, in Table III.

The percentage results do not directly reflect the sampling error as did the previous total weight results, and therefore, despite the closer proximity of the curves, the differences between them are more significant than in the total weights results of Fig. 2. The general level of all constituents, except phosphorus, was again definitely higher in the Edmonton samples. The proportionately greater concentration of phosphorus in the Fallis plants cannot be attributed to the amount of phosphorus in the gray soil, which is only 60% of that present in the black. Nor can it be due to the greater amount of phosphorus in relation to the other elements, because in relation to calcium and magnesium, phosphorus is less in the gray than in the black soil.

The higher level of constituents in Reward was significant for more than 50% of the determinations, the difference between the varieties being in general greater at Fallis for all constituents except nitrogen.

Despite the general similarity of all the curves, the more rapid drop in nitrogen and sulphur of the Fallis plants indicates that these elements were more limiting in the gray soil. During the first month the Fallis plants absorbed approximately 37% of their total nitrogen and 28% of their total sulphur, while the corresponding absorption for Edmonton plants was 19% and 12% respectively. Although phosphorus was definitely higher in the Fallis plants, the percentage of the total uptake of this element absorbed during the first month of growth was 17% at Fallis compared with 16% at Edmonton, indicating that phosphorus was equally available at Fallis and Edmonton.

TABLE III

PERCENTAGE DATA AND THE SIGNIFICANCE OF DIFFERENCES DUE TO SOIL AND VARIETY

Determination	Collection	Mean of 8 replicate samples				*t* values			
		Edmonton		Fallis		Difference between general levels† at Edmonton and Fallis	Difference between varieties††		Difference between varietal reactions at Edmonton and Fallis
		Reward	Red Bobs	Reward	Red Bobs		Edmonton	Fallis	
Dry matter, % green weight	1	13.4	14.6	14.8	15.5	−2.94*	−6.89**	−4.05**	2.01
	2	15.3	16.3	22.8	25.1	−9.13**	−1.35	−3.44**	1.47
	3	21.2	22.2	28.8	30.4	−4.33**	−1.82	−3.13**	0.92
	4	41.8	44.8	44.3	46.7	−2.87*	−3.43**	−2.75*	0.46
Nitrogen, % dry matter	1	5.74	5.50	5.24	5.22	6.55**	3.80**	−0.05	2.90*
	2	4.06	3.85	1.98	1.78	9.36**	2.91*	3.64**	0.52
	3	2.32	2.16	1.12	1.05	12.36**	3.15**	1.29	1.32
	4	1.48	1.33	0.86	0.85	24.97**	6.86**	0.39	4.61**
	5	1.54	1.39	0.99	0.88	17.35**	8.63**	3.80**	3.42**
Ash, % dry matter	1	18.70	16.69	14.14	12.78	12.16**	5.03**	3.70**	0.94
	2	12.28	12.30	8.84	7.80	13.45**	0.38	3.71**	2.35*
	3	8.61	8.14	5.88	5.53	9.34**	2.58*	1.93	0.45
	4	5.70	5.45	5.14	4.60	3.77**	1.25	2.68*	1.01
	5	5.50	4.93	5.88	4.51	0.12	2.03	3.99**	1.39
Phosphorus (P_2O_5), % dry matter	1	1.01	0.85	1.13	1.33	− 4.57**	4.59**	−5.30**	6.99**
	2	0.60	0.61	0.90	0.73	− 2.08	−0.24	3.39**	2.42*
	3	0.40	0.34	0.54	0.51	− 4.80**	4.55**	1.95	1.84
	4	0.28	0.28	0.50	0.42	−11.01**	0.06	4.92**	3.44**
	5	0.26	0.23	0.51	0.46	− 8.89**	1.75	2.91*	0.81
Potassium, % dry matter	1	4.49	4.26	3.64	3.74	3.88**	1.45	0.16	1.14
	2	4.10	4.05	2.90	2.53	12.72**	0.27	2.73*	1.74
	3	2.99	2.77	2.06	1.78	11.51**	3.08**	3.51**	0.30
	4	1.21	1.12	1.06	0.88	5.54**	2.64*	5.38**	1.94
	5	0.78	0.78	0.75	0.65	2.65*	−0.05	3.86**	2.76*
Calcium, % dry matter	1	0.90	0.89	0.73	0.71	5.79**	0.60	1.14	0.38
	2	0.59	0.60	0.37	0.33	9.48**	0.50	1.85	1.66
	3	0.34	0.35	0.20	0.19	17.95**	−0.93	1.24	1.53
	4	0.22	0.28	0.13	0.13	17.68**	−0.98	0.98	1.39
	5	0.22	0.19	0.17	0.14	8.84**	2.56*	3.31**	0.53
Magnesium, % dry matter	1	0.42	0.39	0.30	0.26	9.71**	1.25	1.74	0.35
	2	0.22	0.24	0.11	0.11	18.22**	−1.39	0.56	1.37
	4	0.080	0.060	0.058	0.060	3.46**	4.81**	−0.44	3.71**
	5	0.093	0.071	0.081	0.063	2.64*	2.89*	2.64*	0.17

† *A minus sign indicates that the percentage is higher in the Fallis plants.*
†† *A minus sign indicates that the percentage for Red Bobs is higher than for Reward.*
* *5% point, t = 2.15.*
** *1% point, t = 2.98.*

Various workers (8) have shown that there is a reciprocal relationship between the amounts of nitrogen and phosphorus absorbed by plants and the percentages of these elements occurring within the plant. Low nitrogen supply, together with high available phosphorus, may then be responsible for the definitely higher concentrations of phosphorus in the Fallis plants.

Sulphur is also important in this connection. Marsh (10) has shown that a reciprocal relationship existed between sulphur and phosphorus in the tomato plant. The high concentration of phosphorus in the Fallis samples may then be due in part to the effect of low nitrogen, and in part to the effect of low sulphur.

Ash constituents as a percentage of total ash. The average values for phosphorus, sulphur, potassium and calcium expressed as percentages of the total ash are presented graphically in Fig. 3 and numerically, together with *t* values, in Table IV.

TABLE IV

MINERALS AS A PERCENTAGE OF ASH AND THE SIGNIFICANCE OF DIFFERENCES DUE TO SOIL AND VARIETY

Determination	Collection	Mean of 8 replicate samples				*t* values			
		Edmonton		Fallis		Difference between general levels† at Edmonton and Fallis	Difference between varieties††		Difference between varietal reactions at Edmonton and Fallis
		Reward	Red Bobs	Reward	Red Bobs		Edmonton	Fallis	
Phosphorus (P_2O_5)	1	5.41	5.08	7.98	10.40	−13.15**	1.14	−8.58**	6.87**
	2	4.87	4.93	9.70	9.26	−18.90**	−0.16	1.05	0.86
	3	4.68	4.12	9.17	9.08	−16.87**	2.68*	0.40	1.62
	4	4.98	5.15	9.63	9.20	−15.25**	−0.52	1.30	1.29
	5	4.83	4.73	8.60	10.27	− 7.45**	0.33	−5.39**	4.05**
Potassium	1	24.2	25.5	25.9	29.2	−2.54*	−0.11	−2.78*	1.89
	2	33.5	33.1	32.8	32.4	0.72	0.32	0.36	0.03
	3	34.8	33.9	34.4	32.3	1.38	0.98	2.50*	1.07
	4	21.2	20.5	20.7	19.2	0.72	4.25**	6.91**	1.88
	5	14.4	16.0	12.8	14.5	1.14	−2.43*	−2.57*	0.10
Calcium	1	4.83	5.32	5.33	5.54	−1.59	−2.07	−0.15	1.36
	2	4.76	4.72	4.11	4.27	6.01**	−0.57	−1.17	0.42
	3	3.91	4.32	3.22	3.38	2.61*	−6.80**	−2.63*	2.95*
	4	3.84	4.21	2.25	2.72	10.66**	−2.40*	−0.39	1.42
	5	3.89	3.96	2.94	3.20	7.23**	−0.52	−1.86	0.95

† *A minus sign indicates that the percentage is higher in the Fallis plants.*
†† *A minus sign indicates that the percentage for Red Bobs is higher than for Reward.*
* *5% point, t = 2.15.*
** *1% point, t = 2.98.*

Unlike the previous results the graphs for the individual constituents are not similar. Phosphorus remained fairly constant in the samples from each soil, but was almost twice as high in the Fallis samples. The outstanding difference between the two sets of sulphur curves is again an indication that this element is definitely limiting in the gray soils. Potassium, however, was almost identical for both varieties and soils. While the lower calcium content in the Fallis plants may indicate a lower supply of this element in the gray soil, the slight increase in the last Fallis collection is significant, and is shown in another section of this paper to be reflected in the composition of the grain.

There were no consistent significant differences between the varieties, although there were indications that Red Bobs was higher in sulphur and calcium on both soils. Likewise there were no consistent significant differences between the reactions of the varieties to the two soils.

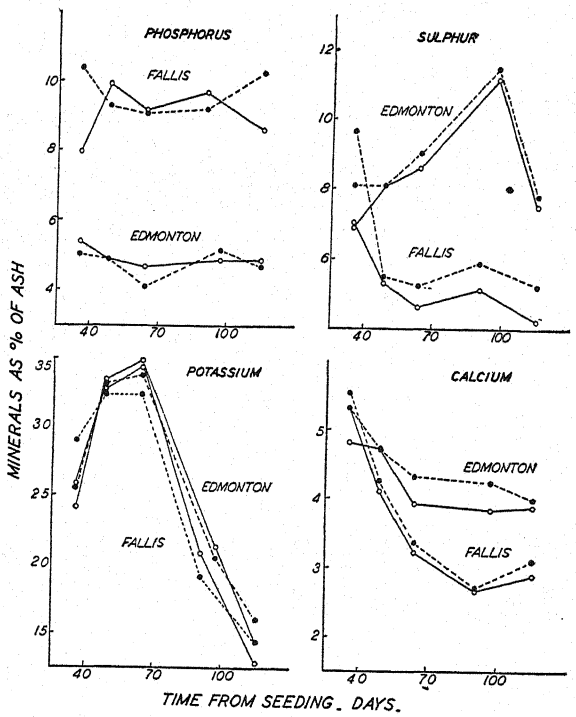

FIG. 4. *Ash constituents as a percentage of total ash in Reward and Red Bobs plants at progressive stages of maturity.*

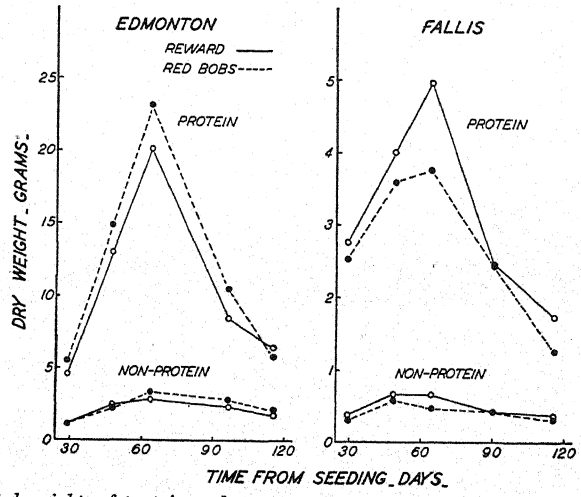

FIG. 5. *Total weights of protein and non-protein nitrogen in the vegetative parts of Reward and Red Bobs from a unit area of soil.*

Nitrogen Fractions

Total nitrogen was separated into protein and organic non-protein fractions. Nitrate nitrogen was also determined in Collection 1, but this fraction was less than 1% of the total nitrogen and is not considered.

The total weights of protein and non-protein nitrogen in the vegetative parts of all collections are represented graphically in Fig. 5. Although the total weights in Edmonton samples were nearly five times as high as those of the Fallis samples, the relative changes in the amounts of total and non-protein nitrogen were in general the same.

Non-protein nitrogen as a percentage of the total nitrogen in the vegetative parts is shown in Fig. 6. The curves overlap only at the time of the second and third collections. As soon as translocation of nitrogen to the grain began there was a larger proportion of the non-protein nitrogen in the vegetative parts of the Edmonton plants due, if the results of comparable water culture experiments are applicable (9), to a proportionally greater nitrogen absorption after heading. This is further substantiated by the fact that the Edmonton plants absorbed on the average 8 gm. of nitrogen after Collection 3, while the Fallis plants absorbed only 3 gm., and is additional evidence that nitrogen was limiting development under Fallis conditions.

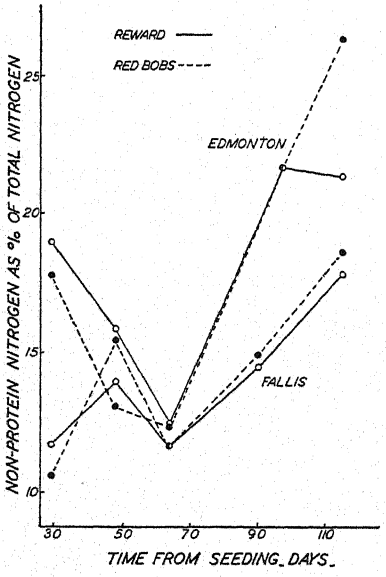

FIG. 6. *Non-protein nitrogen as a percentage of total nitrogen in the vegetative parts.*

Equivalent Uptake of Anions and Cations

The results so far discussed lead to the general conclusion that phosphorus is relatively more available than nitrogen and sulphur in the Fallis soil. The importance of this relationship in altering the composition of the plant is shown in Fig. 7, where the ratios nitrogen : phosphorus and sulphur : phosphorus in the plants at the various collections are plotted. The ratios are calculated from the equivalent weights of the elements, and the graphs show that both of these ratios were definitely higher at all times in the Edmonton plants. This was to be expected if nitrogen and sulphur were limiting at Fallis and phosphorus was readily available.

It has already been suggested that the Fallis plants absorbed large amounts of phosphorus to compensate for the low availability of nitrogen and sulphur. If this reciprocal relation between the anions of nitrogen, sulphur and phosphorus did in some way tend to control the total amount of anions absorbed, then there should be a fairly constant relationship between the principal anions and cations absorbed from each soil. This anion : cation ratio was

calculated in terms of equivalents for the two varieties for all collections and is also shown in Fig. 7. The similarity of the curves for both varieties and soils substantiates the hypothesis that there is a controlling influence which

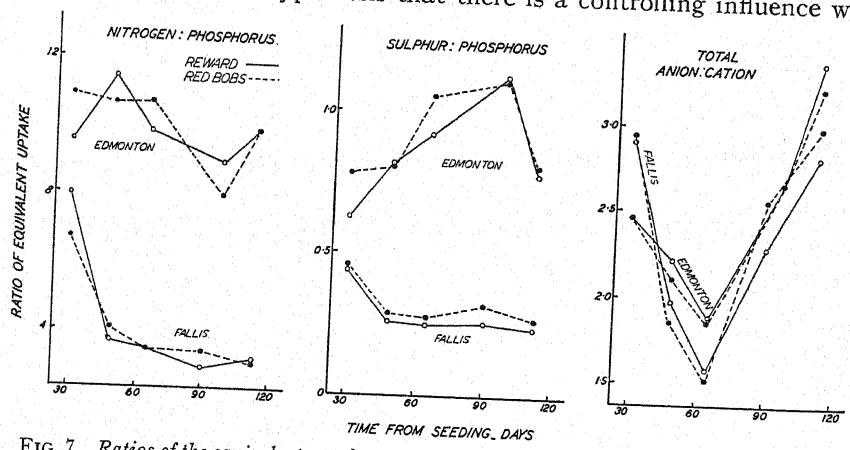

FIG. 7. *Ratios of the equivalent uptake of nitrogen and phosphorus, sulphur and phosphorus, and total anions : cations in Reward and Red Bobs at progressive stages of maturity.*

regulates the proportion of anions to cations absorbed, despite the limiting supply of two of the anions. The high phosphorus content of the Fallis plants was then probably not due to the greater availability of phosphorus in the Fallis soil, but rather to the influences controlling the anion : cation balance.

Percentage Rate of Uptake of Plant Nutrients

Besides the differences in total amounts of nutrients absorbed from the two soils there was a marked difference in the rates of nutrient uptake between collections. It has already been noted that the amounts of nitrogen and sulphur absorbed during the first month of growth were proportionately much greater at Fallis. To illustrate this difference in the rate of uptake the average percentage absorption between collections was calculated. The weight of an element absorbed between successive collections was divided by the number of days between the collections, and this value expressed as a percentage of the total amount of the element absorbed. These percentages for nitrogen, phosphorus, sulphur and potassium, in Reward grown on both soils, are plotted against the number of days from seeding in Fig. 8. The data of this experiment are somewhat inadequate for this type of calculation, but the general shape of the curves clearly demonstrates the point in question.

The increasing rate of nitrogen absorption by the Edmonton plants until Collection 3 indicates that as the plants increased in size their increased nitrogen needs were supplied. The fall in the rate of nitrogen absorption by the Fallis plants means, on the other hand, that there was a limitation of this element at or before the time of the first collection. The somewhat similar relationship between the percentage rates of sulphur absorption can be explained in the same way. The similarity of the phosphorus and potassium curves is evidence that these elements were not seriously limiting in either soil.

It appears that, under the more favorable Edmonton soil conditions, where the supply of these four nutrients can be assumed to be sufficient for excellent growth, the percentage rate of absorption of them by the plant increased until about the time of heading. At Fallis this maximum for the absorption of nitrogen and sulphur was reached at about the time of the first collection, which can only be attributed to the low availability of these two elements in the gray soil.

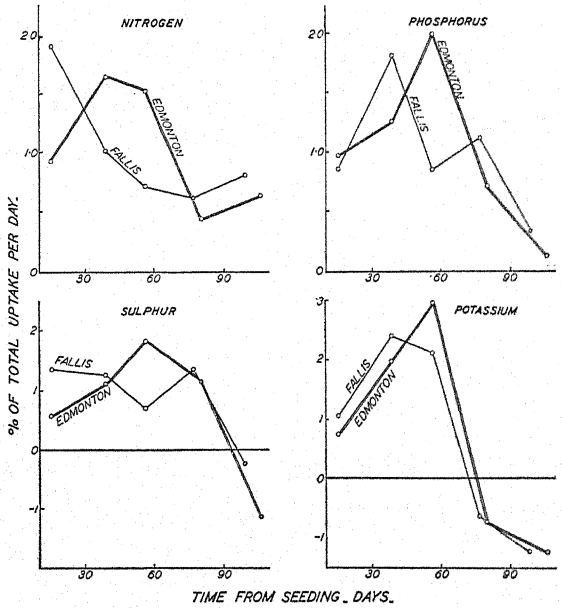

FIG. 8. *The percentage rate of uptake of nutrients by Reward at progressive stages of maturity.*

The foregoing results on the composition of the whole plant have shown the very definite effect of the soil, not only on yield, but also on the composition of the plant. The black soil produces a higher yield of wheat which is richer in all nutrients except phosphorus. The difference in phosphorus is exceptional because as a percentage of dry matter or ash it is significantly higher in the Fallis plants at all stages of maturity, despite the fact that soil analyses show that the total phosphorus in the gray soil is only 60% of that in the black.

Fertilizer experiments (13, 23) have shown that nitrogen and sulphur are limiting in the Fallis soil and the results presented in this paper in every case substantiate these findings. The shape of the total-weight curves, the ratios of equivalent uptake, the percentage rate of uptake, and the low percentage of nitrogen in the Fallis samples, all indicate that nitrogen and sulphur were limiting growth at Fallis early in the development of the plant.

In addition, it was shown that the anion : cation ratio was approximately the same for plants grown on both soils. It is suggested, therefore, that there is a controlling influence which maintains a fairly constant anion : cation ratio

under different nutritional conditions and that under gray soil conditions, where two of the important anions are limiting, the anion : cation ratio is maintained at approximately the usual level by the greater absorption of phosphorus.

The effect of variety in determining the composition of the wheat plant has generally been observed in its effect on the composition of the mature grain or straw. No systematic study of the effect of variety in controlling the composition of the whole plant at progressive stages of maturity has come to the attention of the authors. This study shows that for certain elements expressed as a percentage of dry matter the composition of the two varieties was significantly different at practically all stages of development. Reward was significantly higher than Red Bobs in nitrogen at all collections on the black soil and in total ash, phosphorus and potassium for four collections on the gray soil.

Composition of Straw and Grain at Maturity

Total Weights

The total weights of dry matter, nitrogen, ash and ash constituents in grain and straw of mature plants are shown as bar diagrams in Fig. 9, and numerically together with t values, in Table V.

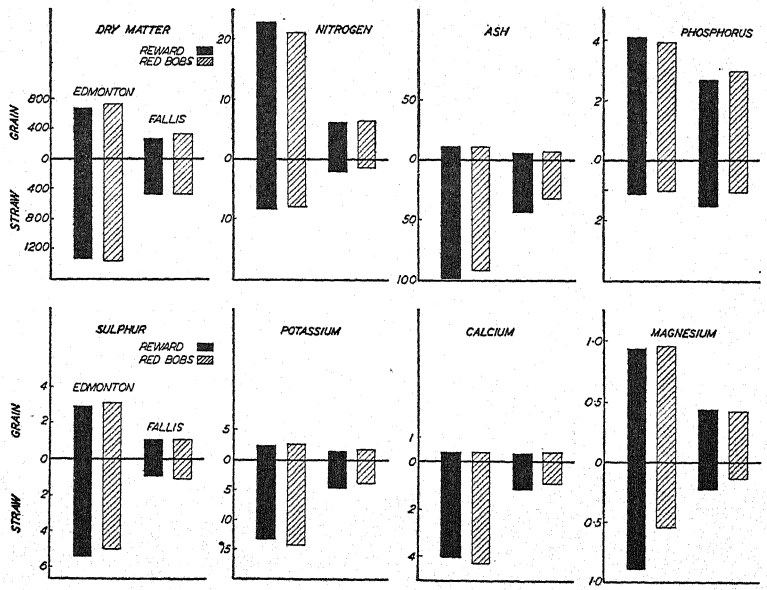

FIG. 9. *Total weight in grams of dry matter, nitrogen, ash, and ash constituents, in grain and straw of the final collection.*

The weights of all constituents were significantly higher in the Edmonton samples except for phosphorus in the straw and calcium in the grain where there were no definite differences between the samples from the two soils. Differences between varieties were significant in only two instances.

TABLE V

TOTAL WEIGHTS IN STRAW AND GRAIN OF MATURE PLANTS AND THE SIGNIFICANCE OF DIFFERENCES DUE TO SOIL AND VARIETY

Determination	Part of plant	Total weight, gm.				*t* values			
		Edmonton		Fallis		Difference between general levels† at Edmonton and Fallis	Difference between varieties††		Difference between varietal reaction at Edmonton and Fallis
		Reward	Red Bobs	Reward	Red Bobs		Edmonton	Fallis	
Dry matter	Grain	675.1	728.6	263.6	324.3	10.24**	−1.35	−1.55	0.14
	Straw	1336.3	1362.0	564.0	560.0	19.55**	−0.30	0.04	0.24
Nitrogen	Grain	22.75	21.06	6.09	6.19	16.00**	1.74	−0.02	1.24
	Straw	8.29	8.04	2.09	1.60	10.98**	0.69	−0.55	0.10
Ash	Grain	11.68	11.95	5.48	6.29	10.80**	−0.02	−0.27	0.20
	Straw	100.49	92.90	44.30	33.73	10.69**	1.41	1.46	0.00
Phosphorus (P_2O_5)	Grain	4.15	3.89	2.66	2.95	4.00**	0.06	−0.97	1.10
	Straw	1.16	1.06	1.58	1.09	−1.28	0.16	3.10**	2.08
Potassium	Grain	2.30	2.55	1.40	1.72	4.61**	−1.52	−1.71	0.14
	Straw	13.50	14.30	4.85	3.98	22.42**	−0.67	0.83	1.05
Calcium	Grain	0.28	0.29	0.26	0.29	0.32	0.03	1.13	0.77
	Straw	4.01	4.60	1.19	0.95	13.75**	0.92	0.84	0.06
Magnesium	Grain	0.95	0.95	0.44	0.42	9.37**	−0.23	0.19	0.29
	Straw	0.90	0.55	0.22	0.14	9.70**	2.52*	0.77	1.26

† *A minus sign means the weight is higher for Fallis plants.*
†† *A minus sign means the weight is higher in Red Bobs than Reward.*
* *5% point, t = 2.15.*
** *1% point, t = 2.98.*

The percentages of nutrients translocated to the grain are shown in Table VI. At Fallis, Red Bobs produced more grain in comparison with total plant than Reward, and there was a greater translocation of all nutrients except sulphur. At Edmonton this difference between the varieties was not so marked, and the percentage of nutrients translocated to the grain was approximately the same for both varieties.

TABLE VI

THE WEIGHT OF DRY MATTER, ASH AND NUTRIENTS IN THE GRAIN AT MATURITY EXPRESSED AS A PERCENTAGE OF THE TOTAL IN THE WHOLE PLANT

Collection		Dry matter	Nitrogen	Ash	Phosphorus	Sulphur	Potassium	Calcium	Magnesium
Edmonton	Reward	33.6	73.2	10.4	78.2	34.6	14.6	6.6	51.4
	Red Bobs	34.8	72.4	11.4	78.5	32.3	15.1	6.7	63.6
Fallis	Reward	31.8	74.4	11.0	62.7	58.9	22.4	17.9	65.9
	Red Bobs	36.7	79.5	15.7	73.0	53.7	30.2	23.1	75.0

The total weights of dry matter and nutrients in 1000 kernels are presented in Table VII. The weight of any element occurring in the grain is dependent upon the total absorption of that element, the percentage of it translocated to

TABLE VII

WEIGHT OF DRY MATTER AND NUTRIENTS PER 1000 KERNELS, AND THEIR RELATION TO NITROGEN

Sample		Dry matter	Nitrogen	Ash	Phosphorus	Sulphur	Potassium	Calcium	Magnesium
Wt. of 1000 kernels									
Edmonton	Reward	26.3	1.007	0.357	0.184	0.129	0.102	0.0126	0.0419
	Red Bobs	26.7	0.876	0.456	0.162	0.130	0.106	0.0121	0.0397
Fallis	Reward	24.1	0.636	0.570	0.277	0.123	0.145	0.0269	0.0452
	Red Bobs	26.1	0.565	0.574	0.269	0.106	0.157	0.0260	0.0385
Nitrogen basis 100									
Edmonton	Reward		100	35.5	18.3	12.8	10.1	1.3	4.2
	Red Bobs		100	52.1	48.5	14.8	12.1	1.4	4.5
Fallis	Reward		100	89.6	43.6	19.3	22.8	4.2	7.1
	Red Bobs		100	101.5	47.6	18.8	27.8	4.6	6.8

the grain, and the yield of grain. Nitrogen was higher in the Edmonton grain because of the proportionately greater absorption of this element by the Edmonton plants, and for similar reasons, phosphorus was higher in the Fallis grain. Sulphur absorption was also higher at Edmonton, but because of the greater percentage translocation of this element to the grain of the Fallis plants the composition of the grain was not greatly altered. The percentage of all cations was higher in the Fallis grain because there was a greater proportion of these elements translocated to the grain.

The outstanding differences in the composition of the various grain samples are shown in the lower section of Table VII, where the weight of each of the constituents is compared with nitrogen expressed as 100. The higher proportion of ash constituents in the Fallis grain is very definite and may be of significance in influencing the quality of the grain (2, 5). The differences between varieties were not so great, but Red Bobs was higher in the proportion of total ash and slightly higher in the proportion of potassium and phosphorus.

Percentage Data

Nitrogen, ash, and ash constituents, as a percentage of dry matter. Nitrogen, ash, and ash constituents of the final collection expressed as a percentage of dry matter are shown in Table VIII, together with accompanying t values.

The higher percentages of nitrogen and all ash constituents, except phosphorus, in the straw of Edmonton plants were in accord with the previous results on the composition of the whole plant. This condition was, however, reversed in the grain where all the constituents, except nitrogen, were definitely higher in the Fallis samples. The exceptionally large amounts of calcium and phosphorus were undoubtedly due to the greater translocation of the former (Table VI) and greater absorption of the latter at Fallis.

TABLE VIII

PERCENTAGE DATA FOR STRAW AND GRAIN OF THE MATURE PLANTS, AND THE SIGNIFICANCE OF DIFFERENCES DUE TO SOIL AND VARIETY

Determination	Part of plant	Mean of 8 replicate samples				*t* values			
		Edmonton		Fallis		Difference between general levels† at Edmonton and Fallis	Difference between varieties††		Difference between varietal reaction at Edmonton and Fallis
		Reward	Red Bobs	Reward	Red Bobs		Edmonton	Fallis	
Percentage of dry matter									
Nitrogen	Grain	3.37	2.89	2.31	1.91	22.37**	50.30**	43.65**	6.12**
	Straw	0.62	0.59	0.37	0.32	5.92**	9.82**	9.88**	1.13
Ash	Grain	1.70	1.57	2.08	1.94	− 6.12**	7.51**	5.02**	1.77
	Straw	7.52	6.82	7.85	6.02	1.01	4.95**	10.42**	3.87**
Phosphorus	Grain	0.61	0.53	1.01	0.91	−18.90**	3.47**	5.09**	1.38
	Straw	0.087	0.078	0.280	0.194	− 8.80**	0.51	4.95**	3.14**
Sulphur	Grain	0.43	0.43	0.45	0.36	—	—	—	—
	Straw	0.41	0.37	0.15	0.18	—	—	—	—
Potassium	Grain	0.34	0.35	0.53	0.53	−15.85**	−0.53	0.19	0.60
	Straw	1.01	1.05	0.86	0.71	5.17**	−0.06	3.84**	2.76*
Calcium	Grain	0.042	0.040	0.098	0.088	− 4.72**	1.80	1.79	0.65
	Straw	0.30	0.28	0.21	0.17	12.07**	2.31*	3.08**	0.55
Magnesium	Grain	0.140	0.131	0.165	0.130	− 2.44*	2.76**	10.45**	5.44**
	Straw	0.067	0.040	0.040	0.025	4.62**	2.95**	1.55	0.99
Percentage of ash									
Phosphorus	Grain	35.5	32.9	48.5	46.8	−10.89**	1.73	1.34	0.65
	Straw	1.16	1.17	3.60	3.21	−14.72**	−0.34	2.24*	1.82
Potassium	Grain	20.0	22.5	25.6	26.8	− 3.65**	−1.53	−0.76	0.55
	Straw	13.6	15.1	11.0	11.9	3.31**	−2.52*	−1.36	0.82
Calcium	Grain	2.48	2.43	4.69	4.57	−10.12**	0.29	0.61	0.22
	Straw	4.02	4.13	2.71	2.91	6.63**	−0.70	−1.28	0.42

† *Minus sign indicates a higher percentage in Fallis plants.*
†† *Minus sign indicates a higher percentage in Red Bobs than Reward.*
* *5% point, t = 2.15.*
** *1% point, t = 2.98.*

Varietal differences were definite, the percentage of all constituents, except potassium at Edmonton, being higher in Reward despite the greater translocation of nutrients to the grain by Red Bobs. The varietal differences were on the whole more definite in the composition of the straw.

The differences between the varietal reactions at Edmonton and Fallis showed that the percentage of nutrients in Reward was higher on both soils, but that this difference between the varieties was often greater at Fallis. Nitrogen in the grain was exceptional, the difference between the two varieties being significantly higher at Edmonton.

Ash constituents as a percentage of total ash. Phosphorus, potassium and calcium expressed as percentages of the total ash in the final collection are also shown in Table VIII, together with their accompanying *t* values.

These results show the same relation between soils as did the previous results expressed on a dry matter basis. The differences between varieties were, however, no longer significant, indicating that the composition of the ash was not altered as much as the composition of the dry matter.

These results show that the soil very definitely influenced the composition of the grain and straw of the final collection. The percentage of all ash constituents, except phosphorus, translocated to the grain was so much greater at Fallis that the composition of the grain became richer and the composition of the straw poorer than at Edmonton. The higher proportion of the calcium translocated to the grain in the Fallis plants resulted in a percentage of calcium twice as high as in the Edmonton grain. Similarly there was a much greater translocation of sulphur in the Fallis plants, but owing to the limiting supply of this element the greater translocation only brought the percentage of sulphur in the Fallis grain to approximately the same level as that in the Edmonton grain. It did, however, markedly lower the sulphur content of the Fallis straw. Translocation to the grain tends to be so regulated that under very diverse nutritional conditions the composition of the grain will be much more nearly uniform than that of the straw, and these results show that the effect of soil or variety in altering the composition of the plant was much greater in the composition of the straw than in the composition of the grain or the whole plant.

The ratio of grain to straw and the proportion of nutrients translocated to the grain determine in a large measure the composition of the grain and influence the quality of the flour into which it can be milled. Red Bobs produced on both soils a greater proportion of grain to straw than Reward, and the difference between the varieties was greater at Fallis. This greater proportionate production of grain by Red Bobs when grown under the less favorable gray soil conditions was accompanied by a greater proportionate translocation of nutrients to the grain, but despite this, the grain of Red Bobs was lower in most constituents and poorer in quality than that of Reward.

Quality of Grain

The quality of the wheats grown in this experiment was determined by the experimental baking procedure using the bromate formula. During 1932 and 1933 Reward and Red Bobs were grown under conditions similar to those of this experiment, and in Table IX the quality of the wheats for the three years is compared by means of protein and loaf volume results.

The differences in loaf volume results show, as has previously been indicated, that Reward was in general higher in quality than Red Bobs at both Edmonton and Fallis. This original quality of the wheats is closely related to protein content, and both loaf volume and protein results show that this relationship between the varieties was, with one exception, the same for all three years.

TABLE IX

Sample		Protein, %			Loaf volume, cc.		
		1932	1933	1934	1932	1933	1934
Edmonton	Reward	16.5	16.8	16.6	708	795	668
	Red Bobs	14.2	14.7	14.2	632	694	545
Fallis	Reward	13.6	12.6	11.4	662	627	515
	Red Bobs	10.3	10.6	9.4	588	632	464

There was, however, a considerable fluctuation in the results from year to year. The 1934 loaf volume results are particularly low in comparison with the 1932 and 1933 results. This can be attributed in part to the climatic conditions of the year, since nearly all wheat grown in central and northern Alberta was of comparatively low quality in 1934, and in part to the effect of a frost on August 22. The frost occurred when the percentage of dry matter in the grain was approximately 58%. Newton and McCalla (14) have concluded that when wheat contains 58% dry matter it has reached a critical stage of development, after which little or no translocation of nutrients takes place. Thus it is assumed that in this experiment translocation to the grain was not affected, although the baking quality of the flour, as indicated by loaf volume results, may have been slightly lowered.

Discussion

The results of the chemical analyses of the developing plants have been expressed in a number of different ways in order that as many differences as possible between varietal and soil reactions might be emphasized. It is unfortunate that the individual total-weight results were not more accurate but the stands of wheat, particularly at Fallis, were not uniform and the samples from each sub-block often varied greatly even though the total weights per collection, representing 48 six-inch sections, undoubtedly were fairly accurate. It was impossible to use bigger samples because of the difficulty in handling in the laboratory but, had the large sampling error been anticipated, the method of sampling might have been improved by collecting a fixed number of plants and then determining the comparative yields of the varieties on the two soils by counting the number of plants per unit length of row.

The importance of soil and variety in affecting the composition of the plant, the quality of the wheat and the keeping properties of the flour has been amply emphasized. Nitrogen absorption and the percentage of protein in the grain are undoubtedly among the main factors in determining the original quality of the grain, but the varietal differences in the deterioration of the flour must be due to other causes, and more detailed studies are necessary before these differences can be definitely related to the differences in absorption and translocation of nutrients already noted.

Wood (21) as early as 1907 suggested that the development of the plant might affect the quality of the proteins produced in the grain. He believed that acids and salts in the cell sap during endosperm formation impress upon the gluten the physical properties that decide the character of the flour obtained from that wheat. Gericke (5) extended this idea, and suggested that the plant sap, even before endosperm formation, might influence directly the quality of the proteins. He has, with other workers (2), stressed the particular importance of minerals in this regard. It is interesting to note that in this particular experiment the ratio of minerals to protein was higher at Fallis and higher in Red Bobs than in Reward, but the relation between nutrition and keeping properties of flour is a different problem from that under discussion by Wood and Gericke.

In the Fallis plants the nitrogen-phosphorus-sulphur balance is very different from that in the Edmonton plants. Since these elements are important constituents of the proteins it is possible that the composition of the Fallis grain was altered in some way which affected quality. If this is the cause, the reason for the much more rapid deterioration of Red Bobs flour is not clear.

The importance of lipoidal substances in affecting the physical properties of the wheat proteins is also known (22). Storage experiments (17) with flour from Edmonton and Fallis wheat have shown that there are greater changes in the lipoidal substances in the Fallis flour, and it seems certain that these changes are related to the deterioration of the baking quality of the flour. Phosphorus is an important constituent of these lipoidal substances, and the greater uptake of phosphorus by plants grown at Fallis may in some way alter the metabolism of these plants so that the particular lipoidal substances formed are more susceptible to alteration than similar substances formed in the grain grown at Edmonton.

The studies reported in this paper have not necessarily shown all of the important differences which may exist in the mineral nutrition of wheat grown on the black and gray soils. The importance of other elements needed in smaller amounts has been increasingly recognized in plant nutrition studies, and it seems equally possible that some of these might influence the keeping properties of flour. Studies on such elements have not been undertaken, but offer possibilities.

At the present time studies on the details of nitrogen, phosphorus and sulphur metabolism appear to be the most promising. Such studies are at present under way.

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FROST-HARDENING STUDIES WITH LIVING CELLS

I. OSMOTIC AND BOUND WATER CHANGES IN RELATION TO FROST RESISTANCE AND THE SEASONAL CYCLE[1]

By J. Levitt[2] and G. W. Scarth[3]

Abstract

The osmotic pressure and non-solvent space of the cells of various types of plant were estimated by the plasmolytic method and related to frost resistance and the seasonal cycle.

Osmotic pressure always rises with hardening and falls with dehardening, and it generally reaches higher values or begins to rise earlier in the hardier species and varieties.

The effect of osmotic pressure in reducing the amount of ice formation is enhanced in woody plants by the condition that only about half the cell volume is occupied by the osmotically active solution. The remainder, *i.e.*, the non-solvent space, is shown to consist partly of bound water and must therefore represent hydrophilic colloid. This occupies an even larger proportion of the sap vacuole than of the protoplasm, and it increases notably with hardening. This change, besides reducing intercellular ice, is regarded as protecting the most vulnerable part of the cell, *viz.*, the vacuole, from being frozen at very low temperatures.

Introduction

The process of hardening against frost produces in extreme cases a remarkable increase in power of resistance. Cells which were sensitive to a few degrees of frost may become capable of enduring −40° or even −80° C. without injury. To account for this, some drastic change in the condition or physiology of the tissues might be anticipated. Yet, while various physical and chemical changes have been noted, the mechanism of frost resistance is still largely a mystery. Perhaps attention has been devoted too exclusively to the properties of tissue extracts and too little to living cells. It is on the latter that we have concentrated, in a series of studies beginning with the present paper. The chief advantages of cell study are these, (i) it is possible to detect local differences in physico-chemical properties not only in individual cells but in different parts of a cell; (ii) it deals with the protoplasmic and other colloids in their natural state—not profoundly altered by death; (iii) it allows investigation of functions and properties which are peculiar to life.

Another distinctive feature of our plan is to use in part cells which are capable of extreme cold resistance, and in which the hardening changes must therefore be greatest. For this reason, we have studied largely the twigs of woody plants, which have no protection in winter.

Review of Literature

It is now a generally established principle that during the hardening period an increase in the osmotic pressure of the cell sap occurs. This is usually due to a hydrolysis of starch to sugar but there may be other causes. Elabora-

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tion of glucosides, such as anthocyanins, has been correlated with cold resistance (40) and, similarly, conversion of disaccharides to monosaccharides. Besides the actual change in the quantity of solute, an alteration in the amount of solvent may be just as effective in modifying the osmotic pressure of the cell sap. Thus, if part of the water is colloidally adsorbed ("bound water"), a change in the water-holding power of the cell, and therefore of its "osmotic pressure", will result. The latter question will be considered in the second half of this paper.

In by far the majority of the papers which have any bearing on osmotic pressure changes, the starch⇄sugar equilibrium is involved.

Russow (37) asserted in 1884 that starch disappears during November and reappears in March. Müller-Thurgau (32) showed in 1886 that exposure of plants (*e.g.*, potatoes) to low temperatures causes a hydrolysis of starch to sugars. However, at this time the consensus of opinion appeared to be that the phenomenon was pathological. It remained for Lidforss (25, 26) to extend and expand their observations. In an exhaustive survey of 130 phanerogams, he was able to show that the winter season causes a complete disappearance of starch from the leaves of evergreens and a corresponding increase in soluble sugars. Several conversions may occur during the winter with changes in temperature. Miyake (30) subdivides Japan into three regions according to the severity of the winter. In the coldest of these, almost all the starch disappears, but in the other two, greater or less amounts are still to be found in overwintering leaves. Specific differences are also well-known (11, 20), as well as variation with the tissue and the character of the cells (41). Even in the severe Canadian winter the pith rays and wood parenchyma of some trees may be packed with starch.

Lidforss (26) further showed that the evergreens are resistant to cold only when their tissues possess these high quantities of sugars. As soon as starch appears in their tissues, frost resistance is lowered. It is but a short step from here to attempt a distinction in the relative winter hardiness of plants by estimating the quantities of sugars occurring in them. Numerous efforts have been made to distinguish in this way between hardy and tender varieties of a species. The group of plants which has served most as experimental material is the winter cereals.

Gassner and Grimme (15) determined the sugar content, and Newton (33) and Martin (28) the depression of the freezing point of the sap of wheat varieties, and found the values to be proportional to their hardiness. Åkerman (1) in particular exhaustively investigated the overwintering ability of wheat varieties in Sweden during a period of five years, and obtained a strict correlation between hardiness so estimated and the quantities of soluble carbohydrates. Osmotic pressure determinations by the plasmolytic method gave similar results. Tumanov (42) proved that light is necessary for the hardening-off of wheat seedlings, and that this is because the young wheat plant must photosynthesize in order to build up a sugar reserve. Dexter (8) found the same relationship with regard to carbon dioxide supply. Govorov

(16) distinguishes between spring and winter wheats on the basis of sugars: the former lose glucose more quickly at warm temperatures, the latter gain it more rapidly at low temperatures. Newton and Brown (34) further state that hardy varieties of wheat maintain their sugar reserves during winter better than the less hardy. Mudra (31) asserts that the relative hardiness of winter wheats can be determined refractometrically, or better by sugar determinations.

This body of evidence from workers the world over (and it by no means exhausts the list) seems to prove beyond all doubt the fundamental importance of soluble carbohydrates to cold resistance in cereals. And yet some contradictory results have been recorded. Salmon and Fleming (38) could discover no relation whatever between hardiness of rye, wheat, barley and oats and their sap density during fall and early winter. Tumanov (42) admits that winter vetch (which, however, is not a grain) is an exception among winter annuals, for it is just as hardy as winter wheat and yet possesses only half the amount of soluble carbohydrates. According to Balde (2), Swedish wheat varieties give sugar values which indicate their hardiness, and some German varieties show a similar trend; but it is not possible to obtain significant results by comparing Swedish and German varieties. Gassner and Goeze (14) could discover no hard and fast series of varieties refractometrically. Constantinescu (7) found that varieties of winter barley of different hardiness showed the same decrease in sugar content and in dry matter when hardened off. Also, less hardy varieties showed a greater total sugar content at low temperatures than more hardy ones.

Results with other groups of plants have, on the whole, not proved quite so satisfactory as with winter cereals. Thus, Chandler (6), after an exhaustive series of experiments with plants which kill at relatively high temperatures, concluded that their killing point is lowered slightly when the sap density is increased. This, however, is not true hardening but merely escaping ice formation or, at best, reducing it to a minimum by the possession of a sufficiently high cell-sap concentration.

Of greater importance are the results with more hardy plants. Gail (13) describes a rapid increase in the osmotic pressure of five evergreens from mid-July to December and January. Meyer (29), however, asserts that the increased osmotic pressure of the pitch pine is insufficient to account for the degree of cold resistance developed. Harvey (17) comes to the same conclusion with regard to cabbage. Hildreth (18) found a strikingly similar increase in sugars from fall to winter in the Duchess and Jonathan apple varieties, and though the former is much hardier than the latter, the difference in sugar content during the winter is small. Non-electrolytes in spruce were shown by Lewis and Tuttle (22) to increase from December to March. In *Pyrola*, on the other hand, there was a steady decrease from December to June. Weimer (44) could find no correlation between hardiness and the freezing point of alfalfa. Rein (35) asserts that the cold death-point does not depend at all on osmotic pressure. But he worked with very dissimilar and

unrelated species, many of which were water plants, and these, Lidforss (26) had shown, do not undergo the same changes of starch into sugar as do land plants.

It is, of course, possible that some of the unfavorable results were due to faulty technique, especially in the method used to extract the juice. Yet the negative data are too numerous to be lightly waved aside in this manner. One is therefore forced to conclude that the relation between osmotic pressure and cold resistance is not always demonstrable. Even Lidforss, the original and most vehement protagonist of the role of soluble carbohydrates in cold resistance, points out that some plants (*e.g.*, the sugar cane and the sugar beet) are quickly killed by light frosts in spite of their high sugar concentrations. On the other hand, he admits that bacteria and mosses are examples of those individuals which are cold resistant without any considerable sugar content.

Recently, some investigators have studied the effect on hardiness of artificial increases in osmotic pressure. Åkerman (1) allowed red cabbage cells to deplasmolyze in a molar solution of erythrite, thus increasing their osmotic pressure about 150%. These cells were able to withstand a temperature 3° C. lower than when untreated. Kessler (1935) similarly allowed cells to take up glycerine but found no change in cold resistance. Iljin (19) repeated and confirmed Åkerman's experiments. Dexter (9) allowed cabbage leaves to take up sugar and obtained an appreciable increase in osmotic pressure and cold resistance. However, the hardiness developed was much less than that accompanying a similar natural increase in osmotic pressure. The same was true in Åkerman's results.

Even here, then, there is agreement among the three investigators who used cabbage seedlings, but disagreement by the one using other species (*e.g.*, *Saxifraga*). At any rate, experiments of this type are not sufficient in themselves. It is first necessary to show that osmotic pressure is correlated with hardening under natural conditions.

With the whole subject in an unsettled condition, more information is obviously needed. In the following investigation a considerable number of plants were tested in the hardened and unhardened condition to determine whether they showed a correlation between cold resistance and the osmotic pressure of the cell sap, and further to ascertain whether this factor was of any importance in differentiating species or varieties with respect to hardiness. Especial attention has been paid to seasonal changes in trees, which, on account of their full exposure to low temperature have to possess a greater power of resistance than herbaceous plants.

Methods

Two methods are practicable for the determination of osmotic pressures in plants, namely the cryoscopic and the plasmolytic. Although the latter yields values that are slightly high, both methods, if properly used, give comparable results. The choice should therefore depend primarily on the type of investigation being conducted.

Yet with few exceptions (1, 26) only the cryoscopic method has been used in cold resistance studies. As a result of this, seasonal determinations have been confined to evergreens, from the leaves of which sufficient juice can be obtained at all seasons. This neglect of deciduous woody plants leaves an important gap in the data. Since part of the following research has been conducted to remedy this deficiency, the plasmolytic method was adopted as most convenient.

Besides eliminating the difficulty of obtaining a proper sample of juice, the plasmolytic method is well suited to this type of investigation in other respects. On account of the small size of the sample required, the same plant can be used for all the seasonal determinations. Samples can also be obtained from the same level in a tree without exhausting the supply. This eliminates the variability that exists between individuals and between different altitudes in each. Furthermore, the corresponding tissue can be tested in all cases, so that there is no danger of mixing one tissue in which starch is stored with another in which it is all hydrolyzed.

The validity of the plasmolytic method as a measure of osmotic pressure is questioned by some. The usual objection is that the solute used may penetrate the cell and so raise its concentration, or it may alter the cellular permeability and allow exosmosis of solutes (3). These criticisms are undoubtedly well founded in the case of potassium salts, which have frequently been used as the plasmolytic agent. In the following investigation, however, calcium chloride was always employed. As shown by Fitting (12) and many others, calcium salts do not penetrate. Scarth (39), in fact, found that calcium prevents the penetration of other ions. It can, therefore, be expected to reduce exosmosis to a minimum. Nevertheless, to be certain, sections were tested before and after a six hour period in calcium chloride. No change in osmotic pressure could be detected.

There still remains the question of the accuracy which can be attained with the plasmolytic technique. Osmotic pressure can be determined cryoscopically to .01 atm.; but this degree of accuracy is fictitious as applied to the plant. Thus, Walter (43), admits that certain errors are involved, and he therefore expresses his results only to the first decimal. Fitting (12) attained the same degree of accuracy with the plasmolytic method. This, however, is possible only with very favorable material. With the cells used in the following investigation, determinations could be made only to 0.01 *M* calcium chloride. The error was not more than about 5%.

Procedure

Sections were first allowed to stain for half an hour in a hypotonic solution of 0.15 *M* calcium chloride containing 5 p. p. m. of neutral red. In the few cases where this proved hypertonic a weaker solution was used. Neutral red is a distinct aid in determining incipient plasmolysis. Since only living cells are stained by this dye, it removes the danger of mistaking dead cells

with coagulated protoplasm for plasmolyzed living cells, or empty cell walls for unplasmolyzed living cells. Furthermore, the slightest incipience shows up sharply.

The calcium chloride is a further aid to clear observation, since it prevents the cell wall from becoming stained. A series of calcium chloride solutions were used, varying in strength from 0.05 molar to molar, and all containing 5 p. p. m. of neutral red. The same solutions remained unchanged for several months. This is an obvious advantage over sucrose or other standard organic substances, both as a time-saver and for the sake of uniformity. Minute growths of fungal hyphae do eventually appear, even in the strongest solutions, but since no nutrient is available the growth is exhausted as soon as the food reserves in the spore are used up. This minute growth cannot affect the concentration or utility of the solutions. From time to time, however, they were checked against other solutions and no alteration in the concentration could be detected. Their constancy was further shown by the fact that with the different series of solutions used throughout a period of four years, unhardened, healthy cabbage seedlings always showed the same osmotic pressure, 0.17 to 0.18 *M* calcium chloride.

The osmotic pressure in atmospheres was calculated from the freezing points (International Critical Tables) by use of the formula

$$\text{O.P.} = 12.06\,\Delta - .021\Delta^2 \quad (23).$$

Osmotic Changes Associated with Hardiness

The plant material investigated is, for the sake of convenience, divided into two groups: (a) Herbaceous plants, (b) Woody plants.

(a) *Herbaceous Plants:* Seedlings were grown in the greenhouse until large enough for use (usually at the age of one to three months). In this state they were, of course, unhardened. These were subsequently subjected to a low temperature (5° C.) in a cold chamber cooled with methyl chloride and having continuous artificial lighting. The latter was supplied by four 200 watt lamps, suspended outside the chamber about two feet above the plants.

The degree of cold resistance developed in cabbage seedlings (variety Jersey Wakefield) by a five-day hardening period was readily determined. Together with a control lot of unhardened plants, they were frozen for twelve hours at temperatures ranging between about −2° and −5° C. All the seedlings were then transferred to the greenhouse and left there for two weeks before estimating the injury (*i.e.*, the percentage of dead leaves). As shown in Table I, the unhardened plants always

TABLE I

RELATIVE FROST RESISTANCE OF HARDENED AND UNHARDENED CABBAGE PLANTS

Age, days	Temp. for 12 hr., °C.	% injury	
		Unhardened	Hardened
60	−1 to −4.5	80	10
49	−1 to −4	95	0
75	−1 to −4.5	100	30
57	−1 to −4	70	15
64	−2 to −6	100	25
73	−2 to −4.5	95	10

suffered between 80 and 100% injury, whereas the hardened ones showed an average of 15%. Each result in the table is an average of nine plants. The 20% injury in the case of the hardened plants was always confined to the lowest one or two leaves.

The time factor is of prime importance in the development of cold resistance. Day by day the plant becomes more hardy until the maximum is reached. This gradual increase offers an ideal opportunity for determining the relation between hardiness and osmotic pressure.

For this purpose, some two dozen potted cabbage seedlings were placed in the cold chamber at 5° C. At daily intervals a leaf was removed from each of six plants and the osmotic pressure determined on longitudinal sections of the petiole. The cells examined were those of the chlorenchyma and pith. These two tissues never differed appreciably in cell sap concentration.

Fig. 1 shows that the osmotic pressure increased steadily during the hardening period.

Two clover varieties—a single cut and a double cut—were similarly hardened and tested for osmotic pressure with the same results. Though the former is supposedly more hardy, it did not exhibit any greater increase in osmotic pressure than the latter (Fig. 1).

Now, cabbage and clover are hardy plants. That is to say, they are able to resist freezing temperatures when in the hardened condition. There are other species, however, which under no conditions will become frost resistant. The question then is: Do the latter show any increase in osmotic pressure as a result of exposure to low temperatures? Sunflower and castor bean seedlings (which belong to this group) were subjected to a temperature of 0° to 5° C. and then tested for osmotic pressure. No change occurred after six days (Fig. 1).

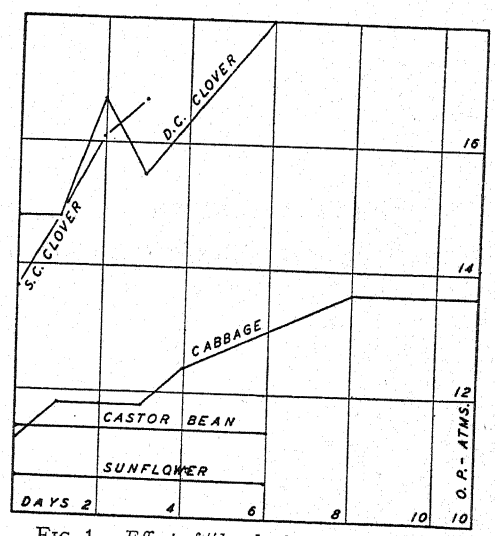

FIG. 1. *Effect of "hardening" temperature (5° C.) on osmotic pressure of hardy (cabbage and clover) and tender (sunflower and castor bean) herbaceous plants.*

(b) *Woody Plants:* Cabbage and clover, though relatively cold resistant, do not overwinter in the province of Quebec unless well protected. The attempt was therefore made to find out whether the very hardy woody plants exhibit changes in osmotic pressure in relation to hardening or dehardening.

Determinations were made on longitudinal sections of the cortex tissue. Terminal growth was always used. The first tests were of a preliminary nature. Twigs were taken indoors during late winter and placed in a beaker

of water for some days. The comparative osmotic pressures before and after the loss of hardiness undergone in this way are shown in Table II. A marked drop is evident in all cases.

An interesting fact about the twigs dehardened in the laboratory is that little or no starch is observable in the cortex cells. Yet the same cells are full of starch when dehardened naturally. Since the twigs taken into the laboratory were very short (about 3 inches long) much less reserves than normal were available for the increased metabolism of the bursting buds, and this caused a considerable drain on the carbohydrates, thus preventing the accumulation of starch which usually occurs on loss of hardiness.

TABLE II

CHANGES IN OSMOTIC PRESSURE OF TWIGS DURING LOSS OF HARDINESS AT ROOM TEMPERATURE

Species	Date	Days at room temp.	O.P.
Hydrangea paniculata	Feb. 23/34	0	27.3
		4	18.6
		7	14.9
		11	14.9
Caragana arborescens	Mar. 14	0	30.2
		15	14.3
Picea pungens	Mar. 16	0	21.2
		11	18.0
		31	13.7
Pterocarya rhoifolia	Mar. 23	0	36.1
		21	19.9
Catalpa hybrida	Apr. 11	0	28.0
		5	14.9
		12	13.0
Aesculus hippocastanum	Apr. 19	0	21.9
		29	12.4

In order to get a more complete picture of the relation between osmotic pressure and hardiness in woody plants, seasonal determinations were made on several species throughout the year. Since they had survived the severe test winter of 1933–34, none of the plants investigated could be considered tender, and consequently it was impossible to make any comparison between species or varieties at the two extremes of hardiness.

Four apple varieties were chosen. Hyslop (a crab) and Hibernal are the two hardiest, and Delicious and Milwaukee the two tenderest grown at Macdonald College. Since no really tender varieties can overwinter there, even the last two possess a considerable degree of hardiness. Further, the individual specimens of these two varieties which furnished the material were necessarily the hardiest of their group, since all the more tender ones had been killed off by the winter of 1933–34. Thus, the Delicious used was the lone survivor in a row of its kind. Almost all the Milwaukee trees had also succumbed. Yet, though these trees were the hardiest in their respective varieties, they had suffered considerably in 1933–34. All the fruit buds and the terminal growth had been killed. They, therefore, did not flower during the spring of 1934, whereas the Hyslop and Hibernal trees both flowered and fruited abundantly. Presumably, this again would tend to reduce the difference in hardiness between the two groups during 1934–35. Finally, the

tissue investigated—the cortex—is the hardiest of all tissues. In spite of all these conditions which tended to reduce the differences in hardiness between the two groups, there can be little doubt that marked differences still existed. Only current growth was used—the kind that killed on the Delicious and Milwaukee in 1933–34 but was unharmed on Hyslop and Hibernal. Determinations were made at intervals of about three weeks throughout the year from October 1934 to October 1935.

Five species of ornamentals were also tested. These were chosen primarily because of the relatively large size of their cortical cells. This facilitated accurate determinations of osmotic pressures. All the native trees examined possessed cells too small for reliable measurements.

The tendency in all cases is the same (Fig. 2)—a minimum O.P. in May and June followed by a gradual rise to a maximum sometime about October,

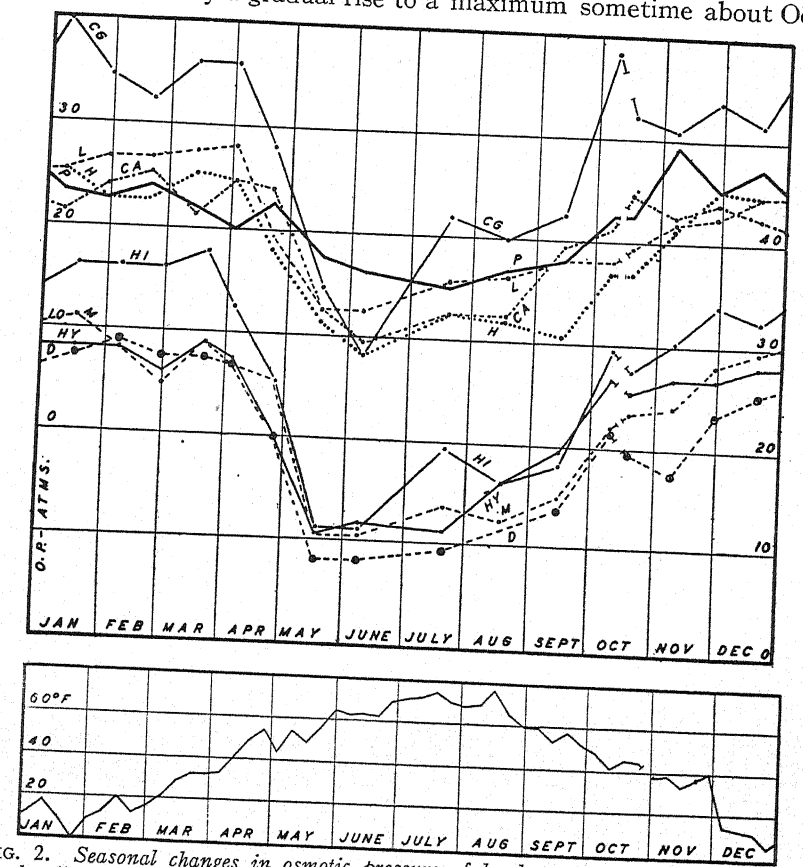

FIG. 2. *Seasonal changes in osmotic pressure of hardy woody plants, determined on cortical cells of current growth, Oct. 1934–Oct. 1935. Lower chart, average weekly temperature.*

Upper Curves

CG = *Caragana arborescens*
L = *Liriodendron tulipifera*
CA = *Catalpa hybrida*
H = *Hydrangea paniculata*
P = *Picea pungens*

Lower Curves (apple varieties)

HI = *Hibernal* } *hardy*
HY = *Hyslop* }

M = *Milwaukee* } *less hardy than above*
D = *Delicious* }

this maximum being retained until April. Here a relatively sharp drop occurs to the minimum in May. The difference between the rate of increase and decrease in osmotic pressure is marked. The gradual rise during summer is obviously primarily due to a slow accumulation of assimilated carbohydrates, though it may be partially caused by drought. (This may account for the sudden mid-July rise exhibited by *Caragana* and Hibernal.) The autumn rise, however, (from mid-September onward) must be mainly if not solely due to a hydrolysis of starch into soluble carbohydrates. Thus, tests for starch during winter were always negative. The sharp drop in osmotic pressure during April is due to a reconversion of the soluble carbohydrates into starch, and this is followed by a further drop to its minimum in May, owing to the drain on the stored carbohydrates caused by the rapid spring growth.

The one evergreen, *Picea*, gives an interesting contrast to the rest. Though it also shows a minimum and maximum, the difference between the two is far less. The maximum is similar to that of the deciduous species but the minimum is about double theirs. Owing to the conifer's ability to photosynthesize just as soon as the temperature rises sufficiently, and also to its less vigorous spring development, the drain on its stored carbohydrates is much less.

The difference between the maxima and minima is astonishingly great. In *Caragana* the minimum is about $7\frac{1}{2}$ atm., the maximum about 40, an increase of over 400%. The extremely high winter osmotic pressure of *Caragana* is interesting in view of its great cold resistance. Judging from its native habitat, it is by far the hardiest of the lot. Not only is its maximum osmotic pressure easily the highest, but it is also reached earliest. In the apples, too, the hardier varieties apparently reach their maximum osmotic pressure sooner than the less hardy. This may indicate an earlier "ripening off" in the case of the more hardy plants, a habit which would be very advantageous in sudden, early "test winters".

During the depth of winter, however, no strict correlation between hardiness and osmotic pressure is evident in the apple varieties. Hibernal, it is true, possesses the highest of all the four. Hyslop, on the other hand, has almost exactly the same osmotic pressure as the two more tender varieties.

Discussion

In the species examined, osmotic pressure is undoubtedly correlated with hardiness. Thus, the eight hardy species tested underwent an increase in osmotic pressure during assumption of cold resistance, whereas the two tender species did not change when exposed to "hardening" temperatures. Furthermore, the two semi-hardy (herbaceous) species had maximum osmotic pressures of only one-third to one-half as great as those found in the six hardy (woody) species. Also among the latter, the hardiest single species possessed the highest concentration and was first to reach its maximum. Among four apple varieties tested the highest osmotic pressure was found in one of the

two hardiest, while the other hardy variety did not differ from the two more tender ones. However, both hardy varieties reached their maxima before the tender ones.

These facts support the view that osmotic pressure is one of the factors involved in cold resistance.

As to its operation, the effect of increased osmotic pressure with regard to freezing is not merely to depress the actual freezing point but also to reduce the amount of ice which forms at temperatures below the freezing point, the latter effect being much the more important as a protection to the cell. It was observed during plasmolysis tests of tree tissues that the reduction in volume of the cells (which is the amount of water withdrawn from them) in solutions of varied strength falls far short of theoretical expectation. The same must apply to the amount of ice which is formed at freezing. In other words, the osmotic pressure of the cells at incipient plasmolysis is not, in such cases, a true measure of their power of resisting ice formation at any and all temperatures. It is necessary to know the osmotic value of the cells at various degrees of plasmolysis in order to correlate temperature with ice development. The next step, therefore, is a study of this relation and of the factors concerned.

The Non-osmotic Fraction of the Cell

If the cell represents nothing more than an osmotic membrane surrounding a solution, then it will obey Boyle's law in as far as that applies to solutions, so that PV will be approximately constant. Thus, if a cell is plasmolyzed by a twice isotonic solution, it should be reduced to half its normal volume. In the case of cabbage this relation was found to hold. Cylindrical cells were chosen and the relative volume of the protoplasts estimated, using the formula: $g = \frac{l - \frac{1}{3}d}{h}$. In both the hardened and unhardened condition the volume of the cell was halved in a twice isotonic solution (Table III). This establishes the fact that the cell sap contains no appreciable amount of solid material that is not osmotically active, and that the force by which it holds water is purely osmotic.

TABLE III

CHANGE IN VOLUME OF CABBAGE CELLS WITH CHANGE IN OSMOTIC PRESSURE
AVERAGE OF 10 CELLS

Seedling	Unhardened		Hardened 4 days		Hardened 7 days	
	Isotonic $CaCl_2$ (mols)	*g in $2i$ $CaCl_2$	Isotonic $CaCl_2$ (mols)	g in $2i$ $CaCl_2$	Isotonic $CaCl_2$ (mols)	g in $2i$ $CaCl_2$
1	0.17	0.50	0.19	0.48	0.20	0.51
2	0.18	0.50	0.21	0.50	0.20	0.48
3	0.16	0.48	0.19	0.51	0.20	0.46
Average	0.17	0.49	0.20	0.50	0.20	0.48

*g = *Höfler's "degree of plasmolysis", i.e., that fraction of its normal volume occupied by the plasmolyzed cell.*

Such an ideal relation is by no means always present. Thus, previous results with onion cells (24) showed that the simple formula $PV = K$ does not hold. It is therefore necessary to use the modified Van't Hoff formula $P(V - x) = K$, where x represents that fraction of the cell volume which does not vary directly with P. From this, $x = \frac{V_1P_1 - V_0P_0}{P_1 - P_0}$.

It is a matter of controversy whether the existence of x in cells is due almost entirely to the presence of non-osmotically active solids or whether colloidally bound (non-solvent) water is also an important factor (27). The question may be decided directly where it is possible to measure accurately the dry weight of colloidal material in the cell. In the case of plant tissues an indirect approach must be used. If x is merely solid matter, its value will be constant for all values of P. On the other hand, if bound water enters into it, then x will vary with P, since bound and free water are in equilibrium. Determinations of x should then be useful in discovering the existence of "bound water" and in comparing its amount in hardy and non-hardy plants.

We have already seen that the value of x is zero, and that therefore there is no bound water in the cell sap of cabbage, whether in the hardened or unhardened state. It is, of course, impossible to generalize from this one case. Furthermore, this tells nothing about the protoplasm itself, since it occupies an insignificant fraction of the large cabbage cell.

An investigation was therefore made on the cortex cells of *Catalpa* twigs in the fully hardened winter condition, and also after they had lost their hardiness by being kept at room temperature. Table IV shows that there is a considerable difference in the value of x, which is 50% greater in the hardened than in the unhardened cells.

TABLE IV

COMPARISON OF x IN CORTICAL CELLS OF HARDENED AND DEHARDENED *Catalpa* TWIGS

	O.P. (atm.)	g in $2i$	x (% of normal volume)
Hardened	18.6	.71	41
Dehardened	11.2	.64	28

Since the osmotic pressures of the hardy and non-hardy cells are not the same, the values of x, if due to bound water, are not strictly comparable, for bound water will vary with the osmotic pressure. The question now is: What is the difference between the values of x when determined under the same osmotic pressures?

To answer this, measurements of cell volume were made in plasmolyzing solutions of different concentrations. Spherical cells were chosen, since cylindrical ones lose their regularity in the very high concentrations here employed. Table V shows the values obtained. The volume of the cell is given both in percentage of its measured volume in 1.0 M dextrose and of its normal volume as found by extrapolation of the volume curve. The value of x is also calculated on these two bases.

TABLE V

COMPARISON OF x IN HARDENED AND DEHARDENED *Catalpa*. AVERAGE OF 16 CELLS

Conc. dex. (mols)	Relative cell vol.				x			
	Hardened		Non-hardened		Hardened		Non-hardened	
	% volume in M dex.	% normal volume	% volume in M dex.	% normal volume	% volume in M dex.	% normal volume	% volume in M dex.	% normal volume
1.0	100	85	100	77				
1.5	83	70	80	62	58	49	50	39
2.0	72	61	69	53	47	40	44	34
2.5	63	53	61	47	41	35	41	32
3.0	61	52	55	42	55	47	38	29

We see that x varies with P and is therefore at least partially due to "bound water". The cold-resistant cells again are characterized by a higher value of x.

Catalpa cells have unusually thick protoplasm layers (Fig. 3) and since the cells are small (compared with cabbage), the percentage of the volume occupied by it is large, about 50%. The possibility suggested itself that the

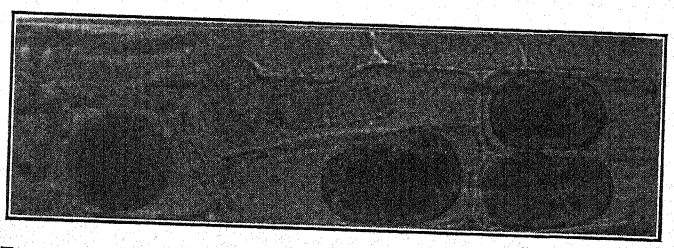

FIG. 3. *Plasmolyzed Catalpa cortical cells showing thickness of protoplasm layer. Vacuole stained with neutral red.*

greater part of x might be localized in the protoplasm. Measurements were therefore made of the thickness of the protoplasm layer in the various concentrations of the plasmolytic agent, as well as of the diameter of the cell as a whole. The relative volumes of x calculated from these measurements are given in Tables VI and VII.

TABLE VI

COMPARISON OF x IN HARDENED AND DEHARDENED *Catalpa* VACUOLES. AVERAGE OF 16 CELLS

Conc. dex. (mols)	Relative vac. vol.				x			
	Hardened		Non-hardened		Hardened		Non-hardened	
	% vac. vol. in M dex.	% normal cell vol.	% vac. vol. in M dex.	% normal cell vol.	% vac. vol. in M dex.	% normal cell vol.	% vac. vol. in M dex.	% normal cell vol.
1.0	100	32.8	100	32.8				
					75	24.5	50	16.6
1.5	90	29.5	80	26.1				
					63	20.5	44	14.5
2.0	82	26.7	69	22.8				
					51	16.6	48	15.8
2.5	73	23.8	63	20.8				
					65	21.2	44	14.5
3.0	71	23.0	58	19.1				

TABLE VII

x IN HARDENED AND DEHARDENED *Catalpa* PROTOPLASM. AVERAGE OF 16 CELLS

Conc. dex. (mols)	Relative prot. vol.				x			
	Hardened		Non-hardened		Hardened		Non-hardened	
	% prot. vol. in M dex.	% normal cell vol.	% prot. vol. in M dex.	% normal cell vol.	% prot. vol. in M dex.	% normal cell vol.	% prot. vol. in M dex.	% normal cell vol.
1.0	100	51.8	100	43.9				
					48	25.0	48	21.0
1.5	79	41.0	79	34.5				
					39	20.5	43	18.7
2.0	67	34.5	68	29.8				
					36	18.5	38	17.0
2.5	58	30.0	58	25.7				
					50	25.5	27	12.3
3.0	56	28.8	50	22.2				

These results show that the difference in x lies not so much in the protoplasm, which might be expected, as in the vacuole.

Seasonal investigations of x were made in *Catalpa* and *Liriodendron*. In both cases, about a 50% drop in x accompanied the natural loss of hardiness which occurs during spring (Table VIII).

TABLE VIII

SEASONAL CHANGES IN x. AVERAGE OF 10 CELLS

Date	*Catalpa*			*Liriodendron*		
	O.P.	g in $2i$ $CaCl_2$	x	O.P.	g in $2i$ $CaCl_2$	x
Mar. 12	22.6	.72	42	25.2	.71	37
Mar. 31	21.9	.70	40	21.9	.68	36
Apr. 20	23.2	.75	52	19.3	.62	24
May 11	14.3	.60	20	17.4	.60	21
June 1	12.4	.65	29	13.0	.53	6
June 22	9.4	.57	13	14.3	.58	16

There is one main difference between cells dehardened in the laboratory and those naturally dehardened. In the former, as previously mentioned, little or no starch appears; in the latter, considerable amounts occur. This would complicate the seasonal change in the value of x. Fortunately, however, *Catalpa* is an exception, for no starch was found in its cortical cells even during June.

It is interesting to compare the theoretical cell volume under a certain degree of frost of hardened and unhardened cells, respectively, as calculated from the osmotic pressure and value of x. The temperature at which unhardened twigs are quickly killed will serve as point of comparison.

Unhardened *Catalpa* twigs (collected in June) were exposed to a temperature of $-6°$ C. for six hours. They were removed from the cold chamber and set in water. Next morning the leaves and young shoot were seen to be dead and blackened. The one-year twig showed injury in two ways:—First, the water in the beaker was colored light yellow, owing to exosmosis from dead cells; second, where the cortex was cut, it was seen to be of an unnatural dark-green color. Sections of the cortex showed that most of the cells were dead, at most only 10% staining with neutral red and exhibiting plasmolysis in a hypertonic solution.

Thus, a temperature of $-6°$ C. was capable of killing most of the cortex cells. Now, since the osmotic pressure of these cells was found to be 9.4 atm. and the value of x, 15%, the following calculations are possible.

$$\text{At } -6° \text{ C., conc. of sap} = \frac{6}{1.86} = 3.22\ M \text{ dextrose}$$

$$\text{Normal conc. of sap} = 9.4 \text{ atm.} = 0.39\ M \text{ dextrose}$$

$$\therefore \text{At } -6° \text{ C., vol. of sap} = \frac{39}{3.22} = \tfrac{1}{8} \text{ of normal}$$

But since $x = 15\%$, only 85% of the cell volume is affected (assuming that x is non-soluble solids and therefore not reduced by the increased pressure).

$\therefore$ Amt. of water removed is at least $= \frac{7}{8} \times 85 = 75\%$ of cell,

i.e., cell is reduced to 25% of its normal volume.

Now the same *Catalpa* cortical cells have a maximum winter value of $x = 40\%$ and an O.P. of 16 atm. Therefore if x were irreducible by ice formation, minimum volume of cell at any temperature would be $\frac{2}{5}$ of its normal volume. But it has been shown that x falls as osmotic pressure rises, the value of 40% in near ($1\frac{1}{2}$) isotonic concentrations being reduced to 25% in 3 M dextrose (approximately the concentration reached at $-6°$ C.).

$$\text{At } -6° \text{ C., then, conc. of sap} = 3.22\ M \text{ dextrose}$$

$$\text{Normal conc. of sap} = 16 \text{ atm.} = 0.66\ M \text{ dextrose}$$

$$\therefore \text{At } -6° \text{ C., vol. of sap} = \frac{.66}{3.22} = \tfrac{1}{5} \text{ of normal.}$$

But since $x = 25\%$ at $-6°$ C., only $\frac{3}{4}$ of the cell volume is affected.

$\therefore$ Amt. of water removed $= \frac{4}{5} \times \frac{3}{4} = 60\%$ of cell,

i.e., cell is reduced to 40% of its normal volume.

At 25% the winter value of x seems to be approaching a minimum. Assuming that further dehydration is not produced at any temperature, then it would be impossible for the cell to contract to the point at which it was killed in the unhardened state—for even below the eutectic point the soluble solids occupy some space to be added to that of insoluble solids and bound water.

Discussion

Osmotic pressure, apart from bound water, was discussed on page 273. An estimation of the non-osmotically active portion of the cell (designated as x) constitutes a valuable method of determining the role of "bound water" in cold resistance. In fact, it is the only technique so far employed on living tissue. Newton's pressure method is in some degree an exception, but it is not a true measure of "bound water" (see 43); other methods cause injury.

Cabbage cells both in the hardened and unhardened condition possess no measurable portion that is non-osmotically active. There is, therefore, no "bound water" in the cell. This is in opposition to the results of investigators (4, 10, 36) who used other methods on the dead products of this plant.

In *Catalpa* and *Liriodendron* cells, on the other hand, the non-osmotically active fraction is large, being greater in the hardened than in the unhardened condition. Furthermore, since the value increases with increase in volume, *i.e.*, water-content, of the cell and since "bound water" increases with free water (within limits), the inference is that x is partially due to "bound water".

A separate estimation of x in the protoplasm and vacuole of *Catalpa* reveals that x, while its actual amount is about the same in both, occupies a relatively larger fraction of the vacuole, and that it is here that the main difference in x between hardened and dehardened cells—at least when dehardened in the laboratory—resides.

During the early part of May, x in *Catalpa* drops to about one-half of its winter value. This drop occurs before any appreciable growth, and therefore it cannot be due to the non-osmotically active solids being used up in growth, as is probably the case later. Another possibility is that these solids may be hydrolyzed to soluble substances, but unless it is counteracted by a concomitant decrease in "bound water" this is unlikely, in view of the marked drop in osmotic pressure coincident with the decrease in x.

The most plausible explanation, then, is that the seasonal drop in the value of x at this stage is due to a decrease in "bound water".

The high mid-winter pressures of woody plants are therefore not merely due to an increase in osmotically active units by hydrolysis of starch to sugar, but are partly caused by an increase in "bound water".

These results give positive evidence of the value of the much disputed "bound water" in cold resistance. However, the usual explanation of its rôle as preventing the dehydration of protoplasm does not appear to hold, for the increase in non-solvent space occurs less in the protoplasm than in the vacuole. When ice forms inside the cell, its site is the vacuole, not the

protoplasm (5). The cell sap is the vulnerable part of the cell and the function of the increase in "bound water" as well as of osmotic substance in the vacuole may be to prevent the freezing of the sap.

But even if ice remains external to the cell, the non-solvent space may be supremely important. Being non-solvent the "bound water" is, of course, valueless in preventing concentration of the sap, which some regard as the immediate cause of frost injury, but it may serve a purpose in reducing the amount of ice formation or in modifying the degree of cell shrinkage, both of which are possible sources of mechanical injury. Our calculations show that at a temperature of −6° C., which is lethal to unhardened *Catalpa* cells, the volume of ice in the tissues is 75% of the original cell volume, while the volume of the cells themselves shrinks to 25%. At the same temperature with hardened tissue the volume of ice is 60% and the cells 40%. Still more significant is the calculation that in the latter, no matter how low the temperature, the ice volume can never rise nor the cell volume fall to those which are reached with fatal effect at only −6° C. in unhardened tissues. This illustrates rather forcibly how important a role hydrophilic colloid in the sap—in conjunction with osmotic pressure—plays in the frost resistance of the cells which have to endure extremely low temperatures; but moderate resistance, as we have seen, is attained without this factor, while its presence in considerable degree in unhardened *Catalpa* cells does not make them as frost resistant as those of hardened cabbage, in which it is immeasurably small. Evidently, other factors are involved and these will be dealt with in subsequent papers.

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FROST-HARDENING STUDIES WITH LIVING CELLS

II. PERMEABILITY IN RELATION TO FROST RESISTANCE AND THE SEASONAL CYCLE[1]

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Abstract

Cell permeability as estimated by the plasmolytic method in various types of plants and for different classes of solute has been studied in relation to frost resistance and the seasonal cycle. Permeability is found to increase greatly with hardening, whether induced by low temperature or other conditions, and it seems to parallel closely the seasonal changes in frost resistance. As regards different species and varieties, cell permeability in the hardened state shows better correlation than any other character with ability to resist frost. The permeability change is greatest towards potassium nitrate—at least in cells (*viz.*, those of hardy woody plants) that are definitely permeable to the salt; the change is more moderate towards polar non-electrolytes with small molecules, such as urea, but with these it occurs in all plants capable of hardening; towards apolar substances, such as urethane, there is no change. These relations point to a widening of the aqueous pores or increased hydration of the plasma membrane as the mechanism of the permeability increase. Hypotheses are put forward as to the means by which freer permeability to water may increase resistance to certain types of mechanical injury by frost.

Many investigators who have attacked the problem of hardiness by the usual physico-chemical methods have been forced eventually to conclude that the seat of the major resistance is the living protoplasm. Indeed, some have regarded their results as proving that hardiness is attended by a protoplasmic change; but the validity of such an inference is doubtful because an alteration in protoplasm can be determined with certainty only from investigation of the living cell itself and not of the products of its disorganization.

On account of the complexity and lability of protoplasm and our lack of knowledge of its true nature, few methods are available for studying its change during life. One of the measurable properties which has received a great deal of attention is permeability. It is moreover a vital property of which post-mortem study tells us nothing. Without any preconceived ideas as to whether or how this might be affected by the hardening process, the following research was undertaken—with surprising results.

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Review of Literature

The possibility that cold injury and resistance to it may in some way be connected with permeability changes has from time to time been suggested. Yet practically no experimental work on the subject has ever been attempted. Lidforss (11) suggested that bacteria and mosses owe their high degree of resistance (in the absence of quantities of sugars) to a ready permeability for electrolytes. In this way, the high concentration of salts which, as Gorke (7) emphasized, must occur in the cell on ice formation, would be prevented by diffusion into the intercellular spaces. The experimental evidence which Lidforss offers is the fact that *Mnium cuspidatum* deplasmolyzes within two hours in a 5.5% potassium nitrate solution, and *Neckera* during 48 hours in 7% potassium nitrate, both of which he considers unusually rapid.

Pantanelli (13) found that cells of the mandarin orange *(Citrus nobilis)*, when subjected to temperatures near freezing, suffer a progressive increase in permeability. Thus, there is a rapid emission of water from the tissue if it is kept dry, or an exosmosis of substances when immersed in water. Bennett (1) recently showed that potato tissue, after being stored at low temperatures, was unable to retain its cellular contents when placed in tap water. The lower the temperature the more rapid was the loss both of electrolytes and non-electrolytes. Golus (6) has recently asserted that hardiness is accompanied by lowered permeability, basing this on the fact that exosmosis from frozen, hardened wheat seedlings was less than that from frozen unhardened ones. All these results have merely to do with changes caused by injury. Death by any method will cause cells to lose their property of semi-permeability, and the larger the number of cells killed the greater will be the increased exosmosis of the affected tissue. Dexter *et al.* (4) have, in fact, made use of this principle in determining the degree of hardiness of plant material. They first subject the plant to freezing temperatures and then determine the quantity of solutes that diffuse into distilled water by measuring its electrical conductivity.

Other suggestions with regard to permeability changes have been made, based mainly on theoretical considerations. Coville (3) believes that the effect of low temperatures in breaking dormancy may be due to an increased permeability of the membrane surrounding the starch grains, thus enabling enzymes to come into contact with them and to convert them into sugars. It has been pointed out that this theory cannot explain the reverse process.

The problem undertaken in the following investigation is to determine just what changes in permeability, if any, occur on exposure to low temperature, and whether hardy and non-hardy plants show any differences. Only when a change has once been definitely established is it in order to consider the theories suggested.

Method and Procedure

For purposes of measurement, cellular permeability may be divided into three types according to the nature of the substances passing in or out:—permeability to *(a)* water, *(b)* non-electrolytes, *(c)* electrolytes. Of the various methods of measuring permeability, the plasmolytic alone is suitable for all three categories of substance and alone is applicable to almost any kind of plant cell. Being also reasonably rapid and capable of a sufficient degree of quantitative estimation, it has proved the most successful in the present study and is the means by which all the positive results were obtained.

In general, our procedure was to compare by means of this method the permeability of the cells of corresponding tissues in the unhardened and hardened state, respectively, and to follow changes as the plant passed from the one phase to the other. The material studied represented various degrees of cold resistance, from tender annuals to hardy trees. The complete list is as follows:

(a) Herbaceous plants:—*Brassica oleracea capitata, Trifolium pratense, Helianthus annuus, Ricinus communis.*

(b) Woody plants:—*Pyrus malus, Hydrangea paniculata, Catalpa hybrida, Picea pungens, Liriodendron tulipifera, Caragana arborescens, Aesculus hippocastanum, Pterocarya rhoifolia.*

The moderately hardy herbaceous species were hardened and tested in a cold chamber. To attain the cold-resistant state the seedlings were exposed to a temperature of 5° C. for five or more days in the presence of artificial lighting. A 12-hour period at about −5° C. was employed to test their hardiness.

The highly resistant trees and shrubs, besides being subjected to artificial conditions, were also examined as they passed through their seasonal changes in a state of nature. Measurements were made principally on the pith and chlorenchyma cells from the petioles of herbaceous plants, and on the cortical cells of woody plants. For purpose of comparison, cells of the same type and position were employed.

The *osmotic value* of the cells was first determined (9). *Permeability* was then studied on the same preparations. Penetration of both water and solutes was measured; and among the latter were examples of various degrees of polarity, from inorganic salts to weakly polar organic compounds. The results are classified under permeability to (1) water, (2) salts (potassium nitrate), (3) polar non-electrolytes (urea, glycerol, etc.), and (4) non-polar substances.

The nature of our problem involved certain complications which in most comparisons of permeability are negligible. Particularly the wide differences between the osmotic values of the hardened and unhardened cells made it impossible to use the same plasmolyzing solutions and also have the cells pass through the same changes in volume and surface in both cases. We have found, however, that the concentration of the plasmolytic has little effect on permeability (see (10) and later in this paper). Hence, without vitiating the experiment, the solution could be varied so as always to give equal degrees of plasmolysis. In the case of water permeability this was the standard procedure and, since cells of about equal size are compared, the absorbing surface should pass through the same area change in both. However, it must be admitted that differences in the amount of non-solvent space will complicate matters still further (9).

In the case of those solutes which penetrate very slowly relative to water, the penetration expressed in *mols* per hour was calculated from the deplasmolysis time and the average difference between internal and external concentration of penetrant. This, of course, is not an exact mode of calculation, but owing to the number of complications and sources of error it seemed difficult and of doubtful value to employ an integration formula on our data. The simple formula used was derived as follows:

Let P = external concentration in mols of penetrant—which remains virtually constant

p = initial osmotic value of the cell, expressed as mols of penetrant

t = time in hours for completion of deplasmolysis

$P.D.$ = average partial pressure (conc.) difference of penetrant (mols)

$$= P - \frac{P-p}{2} = \frac{P+p}{2} \text{ mols} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (I)$$

Assuming that the cell is practically isosmotic with the solution at deplasmolysis, the concentration of penetrant in the cell is then $P-p$. Therefore, as a standard of comparison, the amount of substance which would enter a cell in one hour under a $P.D.$ of 1 mol

$$= \frac{P-p}{t \times P.D.} \text{mols} = \text{(from (1))} \frac{2(P-p)}{t(P+p)} \text{ mols.}$$

This is the value used as an expression of permeability.

In most of the determinations of permeability to *solutes*, differences in degree of plasmolysis were not eliminated, but a preliminary test as well as theoretical considerations seem to indicate that in the type of material used this disturbing factor tends to be inoperative.

In the test, the rate of deplasmolysis of similar cells in solutions of *different concentrations* was measured and permeability calculated according to the above formula, that is without reference to surface area. The results are shown in Table I. The values for each species are nearly constant in spite of great differences in degree of plasmolysis.

In explanation of this result, it is to be noted that in sections such as we used, consisting of a bundle of elongated cells, the effective surface of each and all of the cells in the interior of a section tends on an average to remain the same as deplasmolysis proceeds, for the reason that all the protoplasts expand at the same rate and blanket one another. Thus, whatever concentration of plasmolytic is used, the area of absorbing surface is the same in each case and is virtually constant throughout the test. Accordingly, the results are taken as justifying the application of the formula used. Along with those in Table II they also indicate that varying the concentration of urea does not greatly modify permeability.

TABLE I

COMPARISON OF THE PERMEABILITY RATE IN SOLUTIONS OF DIFFERENT CONCENTRATIONS

Species	Conc. urea, mols	Conc. thiourea, mols	O.P., atm.	Deplas., min.	Perm.,* mols per hr.
Cabbage		0.6	12.4	22	0.44
		0.7		36	0.52
		0.8		60	0.44
Aesculus	0.93		12.4	86	0.41
	1.86			142	0.48
	2.79			165	0.50
Catalpa	0.93		14.9	64	0.38
	1.86			107	0.56
	2.79			151	0.51
Hydrangea	1.86		19.3	27	1.77
	2.79			35	1.90
Picea	1.86		22.5	110	0.36
	2.79			127	0.47

**Calculated per mol difference in concentration.*

An important point to discover is whether it is normal permeability which is measured or an abnormal permeability affected by the mechanical action of plasmolysis or deplasmolysis on the protoplasm. Conceivably it might be resistance to plasmolysis that changes with hardening, rather than permeability *per se*.

The mechanical effect of plasmolysis and deplasmolysis on permeability has been found to vary with the type of cell and its condition at the moment

(5, 8, 14). Accordingly, we tested some of our material that showed the greatest change in permeability. Comparison of penetration of urea in hyper- and hypotonic solutions was made by a modification of the method of Huber and Schmidt (8) on three species in their hardened state.

TABLE II

EFFECT OF PLASMOLYSIS ON PERMEABILITY

Species	O.P., atm.	Time in minutes		Penetration rate, mols per hr.	
		Plasmolyzed	Unplasmolyzed	Plasmolyzed	Unplasmolyzed
		0.8 *M* thiourea	0.4 *M* thiourea		
Cabbage	12.4	60	100	0.44	0.40
		1.5 *M* urea	0.5 *M* urea		
Hydrangea	22.5	52	56	0.54	0.71
Catalpa	22.5	75	90	0.38	0.44

Two parallel determinations were made; one in the ordinary way, the other by use of a hypotonic solution. In the latter case, since no plasmolysis occurred, a special procedure was necessary to find out how much solute penetrated per unit time. Huber and Schmidt (8) determine the increase in osmotic pressure by plasmolysis in dextrose after a definite time in the penetrating solution. In our tests, instead of using dextrose we employed a mixture composed of a partial pressure of calcium chloride equal to the original concentration of the cell sap plus a partial pressure of urea equal to half the concentration of the urea in the hypotonic penetrating solution. A section was from time to time removed from the latter and placed in this composite solution. If the cells plasmolyzed, the section was discarded and a few minutes later another was removed from the hypotonic penetrating solution and similarly tested. This procedure was repeated until the time when no plasmolysis occurred. At this point, the concentration of urea in the cells (which had never been plasmolyzed) was equal to half that in the penetrating solution and exactly equal to the amount in the composite solution. The purpose of this modification of the method used by Huber and Schmidt was to prevent exosmosis of urea during the determination of osmotic pressure. In the case of cabbage, thiourea was employed.

The results are given in Table II. Permeability was found to be practically unaffected by plasmolysis in all three species. Apparently, then, our deplasmolysis tests, in the case of solutes like urea at least, are a measure of normal permeability. On the other hand, the more rapid deplasmolysis involved in the usual test of *water penetration* does produce a temporary increase of permeability (10). Differences in the operation of this effect have to be taken into account in making comparisons of water permeability (see later).

Results

1. *Permeability to Water*

Cabbage seedlings were hardened in the cold chamber in the usual way and determinations of water permeability made from time to time. For reasons explained above, the standard procedure was to plasmolyze for 15 to 20 minutes in twice isotonic dextrose and then to deplasmolyze in an isotonic solution of the same substance. The values given for relative permeability take account of the pressure difference as well as deplasmolysis time.

TABLE III

WATER PERMEABILITY OF CABBAGE SEEDLINGS AT DIFFERENT STAGES OF HARDENING. EACH VALUE AN AVERAGE OF 3 PLANTS

Hardening, days	O.P., atm.	Deplasmolysis time, min.	Relative permeability*
0	10.6	15	2.1
3	12.4	8	3.4
7	16.1	5	4.1
10	16.1	5	4.1

*$Relative\ permeability = \frac{10{,}000}{t\ secs. \times av.\ P.D.}$

Table III shows that the water permeability increases on hardening until the end of a week, and then remains constant. It is then double that of the unhardened plants.

Woody plants. Fully hardened twigs were taken indoors during midwinter and then left standing in water at room temperature for a few weeks. Table IV shows that during this time—that is, in the period when hardiness was lost—a marked decrease in permeability to water occurred.

TABLE IV

WATER PERMEABILITY BEFORE AND AFTER LOSS OF HARDINESS. PLASMOLYZED 15 MINUTES IN $2i$ $CaCl_2$; DEPLASMOLYZED IN $\frac{1}{2}$ $CaCl_2$. JANUARY 16, 1935

Days at room temp.	*Hydrangea*			*Catalpa*		
	O.P., atm.	Deplas., sec.	Rel. perm.	O.P., atm.	Deplas., sec.	Rel. perm.
0	23.9	20	21	22.5	35	12.7
37	11.2	120	7.5	16.1	85	7.3

Experiments with woody plants under natural hardening conditions were begun before standardization of the procedure. In this case the cells were plasmolyzed for 15 minutes in a $2M$ dextrose solution and deplasmolyzed in a $M/2$ dextrose solution (except in *Caragana*, for which, on account of its high cell-sap concentration, double strength was used, *i.e.*, M dextrose). But since the change in osmotic pressure was small during the period when the determinations were made, the differences in degree of plasmolysis were relatively slight. Relative permeability was calculated as before. Table V indicates a definite increase in permeability to water during the late fall. From then on, the rate remained constantly high—that is, deplasmolysis was so rapid as to be immeasurable by the technique used.

TABLE V

WATER PERMEABILITY OF WOODY PLANTS DURING DIFFERENT STAGES OF NATURAL HARDENING. PLASMOLYZED 15 MIN. IN 2*M* DEXTROSE; DEPLASMOLYZED IN $\frac{M}{2}$ DEXTROSE, EXCEPT *Caragana* WHICH WAS DEPLASMOLYZED IN *M* DEXTROSE

Date	*Picea*			*Liriodendron*			*Caragana*			*Catalpa*			*Hydrangea*		
	O.P., atm.	Depl., sec.	Rel. perm.	O.P., atm.	Depl., sec.	Rel. perm.	O.P., atm.	Depl., sec.	Rel. perm.	O.P., atm.	Depl., sec.	Rel. perm.	O.P., atm.	Depl., sec.	Rel. perm.
Oct. 10	21.2	210	2.0	18.6	80	5.6	32.3	60	10.3	31.6	300	1.2	16.8	360	1.3
Oct. 31	28.7	30	12.1	21.9	60	6.9	29.5	40	16.9	26.6	45	8.4	20.6	180	2.4
Nov. 21	25.2	60	6.5	21.2	30	14.0	31.6	30	21.1	22.5	180	2.3	22.5	270	1.5
Dec. 12	24.6	30	13.1	23.9	30	13.3	29.5	*	*	22.5	60	6.9	20.6	150	2.9

**Too rapid for measurement.*

These preliminary tests indicate that in order to measure the water permeability of fully hardened cells, some method of slowing up the rate of deplasmolysis is essential. Since permeability of *Catalpa* cells was found to have a temperature coefficient of about 2.1 (Table VI), an obvious way of reducing the rate was to lower the temperature at which the test was conducted.

TABLE VI

TEMPERATURE COEFFICIENT OF WATER PERMEABILITY OF *Catalpa* CELLS

	O.P.	Deplasmolysis time (secs. from $2i$ to $\frac{i}{2}$ $CaCl_2$)			Temperature coefficient	
		22° C.	10° C.	0° C.	0–10° C.	10–22° C.
Hardened	18.6	40	105	225	2.14	2.20
Unhardened	11.2	100	255	525	2.06	2.13

For this purpose, a special low-temperature stage was made, by means of which the permeability could be measured at 0° C. Fig. 1 shows its construction.

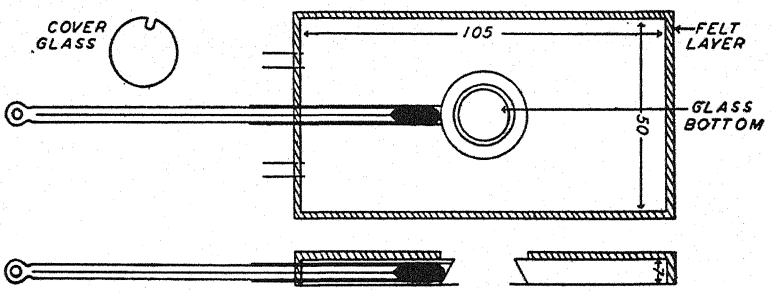

FIG. 1. *Low temperature stage used for measuring water permeability at 0° C. Dimensions in mm.*

The permeability was then measured at intervals from March to June. The standard procedure of plasmolyzing the cells in $2i$ dextrose and deplasmolyzing in $\frac{i}{2}$ dextrose was followed. There was no pretreatment with neutral red and calcium chloride, for preliminary tests showed that this causes a marked decrease in permeability. In other words, the true rate was found to be actually greater than the high values already recorded! Furthermore, the difference between hardy and non-hardy cells is undoubtedly greater than that already found. Thus, Lucké and McCutcheon (12) demonstrated the same retarding effect of calcium, and they further found that this effect only occurred in the case of cells with high permeability and not with those already having a naturally low rate.

Table VII, recording seasonal change, shows a decided drop in permeability during spring. In March and April the rate of deplasmolysis is so rapid as to be practically instantaneous, even at 0° C. In May, however, the time required is a minute or more.

TABLE VII

SEASONAL DETERMINATIONS OF PERMEABILITY TO WATER AT 0° C. DEPLASMOLYSIS TIME (SEC.) FROM 2*i* DEXTROSE TO $\frac{i}{2}$ DEXTROSE

Date	*Hydrangea*	*Catalpa*	*Caragana*	*Liriodendron*
Mar. 12	30	60	30	30
Mar. 31	15	75	20	20
April 20	15	60	15	30
May 11	60	180	60	90

However, a troublesome though interesting complication arose in these investigations. During April and May, as the plants lost in hardiness, it became increasingly difficult to distinguish deplasmolyzing cells, owing to the fact that more and more of the cells burst during deplasmolysis. A test conducted on June 1st was a failure, since all the cells burst before deplasmolysis was complete (except in the case of *Catalpa*). Yet the same non-hardy cells were for the most part uninjured when the deplasmolysis was carried out at room temperature, in spite of the fact that permeability and therefore deplasmolysis rate is about five times as great at the higher temperature! This indicates that the viscosity of the protoplasm is considerably increased by the decrease in temperature—presumably more so in the unhardened than in the hardened cells in view of the lack of injury to the latter during deplasmolysis. This question will receive treatment in a subsequent paper.

Since the permeability of the cell is increased during deplasmolysis, owing to stretching of the membrane (10), the result is to over-estimate the natural permeability. And since the deplasmolysis injury is far more pronounced in unhardened cells, the over-estimation will be greater in the case of these, and, consequently, the true difference in permeability between hardened and unhardened cells is probably much larger than the tests indicate.

On account of the complications involved in the methods so far used, the relation between water permeability and hardiness is not yet completely worked out. Some more satisfactory procedure will have to be evolved in order to get accurate results. In the meantime, we have followed more intensively the penetration of solutes, accurate measurement of which is more easily made.

2. *Permeability to Salts (Potassium nitrate)*

While no evidence of penetration of calcium chloride was found under any conditions, results were obtained with other salts. Potassium nitrate was chosen for the comparative studies.

With cabbage cells the rate of entry of potassium nitrate was too slow to give reliable results. Woody plants in their winter state, however, proved remarkably permeable to the salt, and it was found that after twigs of such

had been kept seven days at room temperature, so as to become partially dehardened, the permeability fell to a small fraction of its original level (Table VIII).

TABLE VIII

PERMEABILITY TO KNO_3 OF TWIGS BEFORE AND AFTER LOSS OF HARDINESS

Twigs collected February 6, 1935. (*Picea* in .75*M* KNO_3)

Species	Days at room temp.	O.P., atm.	Deplas., in 1.25*M* KNO_3, min.	Permeability, mols per hr.
Hydrangea	0	22.5	39	1.03
	7	19.3	73	.68
Catalpa	0	22.5	39	1.03
	7	21.9	215	.20
Caragana	0	34.6	16	0.69
	7	21.2 >	720	< .06
Liriodendron	0	25.9	7	4.50
	7	23.9	106	.36
Picea	0	21.2	5	3.27
	7	23.2	58	.67

Seasonal changes in potassium nitrate permeability. These results were followed up by a seasonal determination of potassium nitrate permeability on five ornamental plants and four apple varieties throughout the year. Unfortunately it was not realized at this time how greatly the prestaining treatment of sections in solutions containing calcium chloride reduced permeability. Consequently, all the results here recorded

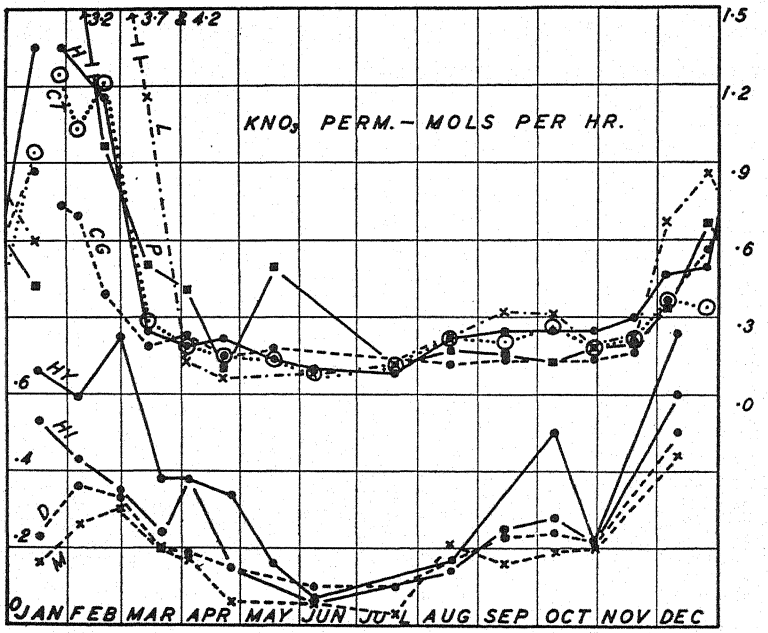

FIG. 2. *Seasonal changes in potassium nitrate permeability of hardy woody plants, determined on cortical cells of current growth. Jan. 1935–Jan. 1936.*

Upper Curves

CG = *Caragana arborescens*
L = *Liriodendron tulipifera*
CA = *Catalpa hybrida*
H = *Hydrangea paniculata*
P = *Picea pungens*

Lower Curves (apple varieties)

HI = *Hibernal* } *Hardy*
HY = *Hyslop* }

M = *Milwaukee* } *Less hardy*
D = *Delicious* } *than above*

are too low, higher rates of penetration being undoubtedly most reduced. Furthermore, fluctuations occur, since they are not all reduced to the same extent, for this will vary with the length of time in the calcium chloride solution. Nevertheless, the seasonal changes are striking (Fig. 2). The maximum occurs in all species from December to February, the minimum from May to July. Furthermore, the maximum may be many times as great as the minimum. The fact that non-solvent space x (see (9)) was not taken into account in our formula cannot greatly influence these results, for even if x is assumed to be 40% in the hardy and 0% in the non-hardy cells, our maxima will only be overestimated about 67%.

As regards varietal difference, Fig. 2 shows that during winter the two hardy varieties (Hibernal and Hyslop) have much higher permeabilities than the more tender ones (Milwaukee and Delicious). It is interesting to note that of the two hardy varieties, Hyslop possesses the higher permeability, whereas Hibernal has the advantage in osmotic pressure, hardiness being about the same in both.

As a further test, ten varieties of varying hardiness were chosen, and potassium nitrate permeability determined three times between mid-January and mid-February. Table IX gives the average of these determinations. It is interesting to note that in spite of the correlation exhibited between hardiness and permeability, osmotic pressure appears to be unrelated to either. The relative hardiness of the varieties was obtained from the observations made by Dr. R. F. Suit of Macdonald College on the injury caused by the test winter of 1933–34.

TABLE IX

VARIETAL FROST RESISTANCE OF APPLES AS RELATED TO KNO_3 PERMEABILITY. AVERAGE OF 3 DETERMINATIONS

(Hibernal and Hyslop average of 2)

Group (increasing hardiness)	Variety	O.P., atm.	Perm., mols per hr.
1	Milwaukee	28.7	0.23
	Delicious	28.0	.31
2	Alexander	32.3	0.33
	Fameuse	25.2	.37
	McIntosh	29.4	.30
	Wealthy	25.9	.42
3	Wolf River	36.8	0.43
	Patten Gr.	27.3	.53
4	Hibernal	36.1	0.49
	Hyslop	28.0	.66

3. *Permeability to Polar Non-electrolytes*

Two extreme types of non-electrolytes were tried: (1) relatively polar compounds with small molecules which, according to the lipoid-sieve theory of the plasma membrane, pass like water and electrolytes through the pores, and (2) apolar compounds with large molecules which are lipoid-soluble and pass through the substance of the membrane.

As examples of the former, thiourea, urea or glycol (molecular weights 76, 60 and 62, respectively) were used as was found convenient, depending on the permeability of the material. Although hardiness is accompanied by an

increase in osmotic pressure of only about 20% (Levitt and Scarth (9)), permeability to thiourea and urea rises 300%. On the other hand, those species incapable of hardening (*i.e.*, sunflower, castor bean) exhibited no

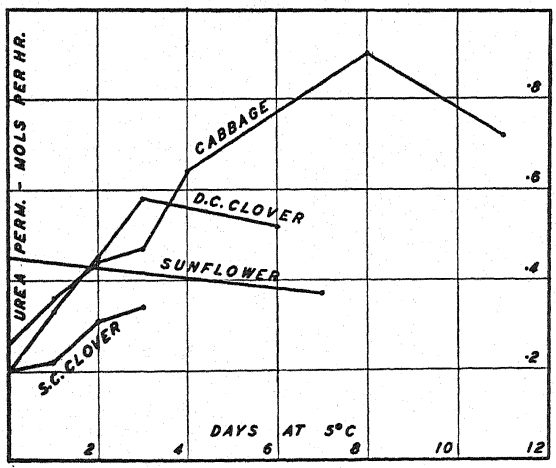

FIG. 3. *Effect of "hardening" temperature on the permeability of hardy (cabbage and clover) and tender (sunflower) herbaceous plants to urea (clover) and thiourea (cabbage and sunflower).*

change either in osmotic pressure or in permeability, on exposure to hardening temperatures. The same change occurs no matter what particular polar substance is used (Table X).

TABLE X

PERMEABILITY OF HARDENED AND UNHARDENED CABBAGE SEEDLINGS (CHLORENCHYMA CELLS) TO THIOUREA AND GLYCOL

	O.P., atm.	Deplas., min.		Perm., mols per hr.	
		Thiourea, 0.75 *M*	Glycol, 0.75 *M*	Thiourea, 0.75 *M*	Glycol, 0.75 *M*
Unhardened	10.6	60	13	0.52	2.40
Hardened	13.6	15	3½	1.16	5.00

Twigs of woody plants in their winter state were tested for urea permeability before and after being kept in the laboratory for a number of days. Table XI shows that there is a decrease in all cases. The final reading ranged from 1/2 in *Picea* to 1/11 in *Catalpa*, of the original. The greater and more rapid the decrease, the earlier did the buds open. This suggests a possible relation of permeability to dormancy, but a study of seasonal changes does not educe a very good correlation in this regard.

Seasonal changes in urea permeability. As with potassium nitrate, tests were made throughout the year. Here again the minima always occurred when the plant was tenderest to frost (May-July) and the maxima when most resistant (Fig. 4). The extremely high winter values for *Liriodendron* and

TABLE XI

CHANGE IN OSMOTIC PRESSURE AND PERMEABILITY OF TWIGS KEPT AT ROOM TEMPERATURE

Species	Date	Days at room temp.	O.P., atm.	Conc. urea, mols	Deplas. time, min.	Molar penetration per hour
Hydrangea paniculata	Feb. 23/34	0	27.3	1.86	30	1.01
		4	18.6	1.4	90	.39
		7	14.9	.93	60	.40
		11	14.9	.93	55	.44
Caragana arborescens	Mar. 14	0	30.2	2.79	40	1.15
		15	14.3	.93	145	.19
Picea pungens	Mar. 16	0	21.2	2.79	120	.52
		11	18.0	.93	30	.46
		31	13.6	.93	125	.24
Pterocarya rhoifolia	Mar. 23	0	36.1	2.79	30	1.20
		21	19.9	1.86	40	1.16
Catalpa hybrida	April 11	0	28.0	2.79	16	3.12
		5	14.9	.93	64	.38
		12	13.0	.93	111	.29
Aesculus hippocastanum	April 19	0	21.9	2.79	21	2.93
		29	12.4	.93	86	.41

Catalpa are not comparable to the others, for they were based on measurements of "protoplasmal deplasmolysis". In other words, an ultra rapid penetration of the outer membrane occurred far in advance of the penetration through the tonoplast. In some cases there is another maximum during August, but, as we shall see later, drought resistance is also correlated with urea permeability, and this may be the explanation. By September, all the values have risen considerably from the spring minimum, whereas in the case of potassium nitrate permeability the initial rise occurs about November. Furthermore, the first drop in permeability to potassium nitrate is registered at the end of March—a full month ahead of the decrease in urea permeability.

As for varietal resistance, the results are not so clear-cut as with potassium nitrate permeability. However, during the winter months the two hardy apple varieties do show a higher permeability than the more tender ones. Since increased urea permeability apparently parallels the early stages of hardening, it is not surprising to find it less strikingly correlated with varietal hardiness than potassium nitrate permeability.

Assuming that the change in rate of penetration is due to alterations in the pore size of the plasma membrane, then it appears that the initial increase in diameter allows more rapid entrance of urea but not of potassium nitrate. Any further enlargement enables potassium nitrate to penetrate more readily. The summer drought was therefore sufficient to speed up urea permeability, but only when full pore size was attained (during winter) did potassium nitrate permeability increase. Furthermore, as soon as the height of the

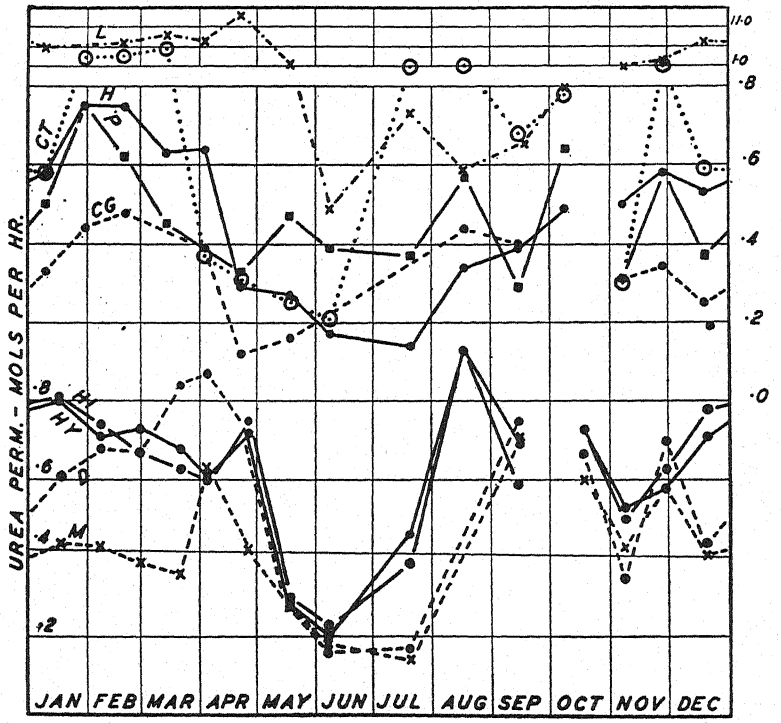

FIG. 4. *Seasonal changes in urea permeability of hardy woody plants, determined on cortical cells of current growth. Oct. 1934–Oct. 1935.*

Upper Curves
CG = Caragana arborescens
L = Liriodendron tulipifera
CA = Catalpa hybrida
H = Hydrangea paniculata
P = Picea pungens

Lower Curves (apple varieties)
HI = Hibernal, *HY = Hyslop* } *Hardy*
M = Milwaukee, *D = Delicious* } *Less hardy than above*

winter began to recede, the decrease in pore size was mirrored in a reduced potassium nitrate penetration rate, but only later were the pores sufficiently reduced to cause a drop in urea permeability.

4. *Permeability to Apolar Substances*

From the list of substances used by Collander and Bärlund (2), urethane coming at the top of their list and succinimide some way down the series were chosen. Only comparative permeability could be tested on account of their quick penetration, which necessitated using the technique of finding the lowest concentration causing plasmolysis. Also, as these concentrations of the pure substance were high and toxic, mixed solutions of the test substance and of calcium chloride having a partial osmotic pressure equal to that of the cell sap had to serve the purpose. Table XII gives the results of an experiment in which hardening, while it induced osmotic pressure to increase 32% and thiourea permeability 100%, affected the penetration rate of urethane and succinimide not at all.

An attempt was also made to determine the permeability of hardy and non-hardy cells to dextrose. Since this substance penetrates cells very slowly, if at all, the method adopted was such as to detect even very slight penetration. Sections of hardened and dehardened *Catalpa* were placed in isotonic solutions of dextrose, so that about half the cells showed incipient plasmolysis. These cells were then examined for deplasmolysis from time to time, but neither hardened nor dehardened cells showed any change even after 24 hours. At the end of 48 hours those cells still alive had deplasmolyzed, but this was obviously due to injury.

TABLE XII

PERMEABILITY OF HARDENED AND UNHARDENED CABBAGE SEEDLINGS (CHLORENCHYMA CELLS) TO POLAR AND APOLAR SUBSTANCES

	Unhardened	Hardened
O.P.	10.6 atm.	14.3
Thiourea perm.	.82 *M* per hr.	1.64

PARTIAL CONCENTRATION CAUSING INCIPIENT PLASMOLYSIS

Urethane	.50 *M*	.50 *M*
Succinimide	.05 *M*	.05 *M*

Collating our results with electrolytes, polar non-electrolytes and apolar non-electrolytes respectively, we find that the maximum effect of hardening on permeability is greater the more polar the compound, and no effect is shown with apolar or lipoid-soluble substances. We note also that the seasonal rise in permeability appears first in the case of urea, etc., and much later in the case of salt. The seasonal fall in spring occurs in the reverse order. If the permeability change is due to change in pore diameter, the inference is that potassium nitrate requires wider pores than urea.

Relation of permeability to osmotic pressure. Most of our records show a parallel variation of osmotic pressure and permeability, but there is also plenty of evidence to prove that the connection is not a necessary one. For example, the osmotic pressure of *Helianthus* was found to increase 80% from an early stage of growth to the flowering period, while permeability remained unchanged (Table XIII).

Helianthus is incapable of hardening, but an experiment with cabbage, in which cut leaves were allowed to

TABLE XIII

OSMOTIC PRESSURE AND PERMEABILITY OF *Helianthus* AT DIFFERENT STAGES OF GROWTH

Age, days	O.P., atm.	Deplasmolysis, min. in .75 *M* thiourea	Permeability, mols per hr.
54	10.6	70	0.45
80	12.4	50	.46
89	14.3	32	.45

TABLE XIV

EFFECT OF SUGAR FEEDING ON OSMOTIC PRESSURE AND PERMEABILITY OF CABBAGE LEAVES (CHLORENCHYMA CELLS)

(Average of 3 plants)

Days in $\frac{M}{5}$ dextrose	O.P., atm.	Conc. thiourea, mols	Deplas., min.	Permeability, mols per hr.
0	10.9	0.75	42	0.71
2	13.0	1.00	60	.60

take up dextrose solution until the osmotic pressure of the chlorenchyma cells rose 20% also gave no increase but rather a slight decrease of permeability as a result (Table XIV).

Increase of frost resistance in such a case is slight, being merely proportional to the depression of the freezing point and not comparable to the increase which is associated with an equal osmotic change combined with a rise in permeability as produced by normal hardening. These experiments were confined to urea permeability, but we have seen with regard to electrolyte permeability that its seasonal rise and fall does not synchronize with change in osmotic pressure.

Relation of Permeability to Hardiness Induced Otherwise Than by Cold

Wilting. Cold resistance as well as drought resistance of plants is increased by restriction of their water supply. It is interesting to know whether the development of drought resistance in a typical xerophyte is attended by any permeability change. *Spartium junceum*, a switch type of xerophyte, was kept unwatered for two weeks. During the second week, wilting began and progresssed greatly, osmotic pressure rose 35%, and permeability was almost doubled (Table XV).

TABLE XV

OSMOTIC PRESSURE AND PERMEABILITY CHANGE DURING INCREASE OF DROUGHT RESISTANCE OF *Spartium*

Days unwatered	O.P., atm.	Deplas., min.	Conc. urea, mols	Urea perm., mols per hr.
0	18.0	66	1	0.27
7	17.4	55	1	.37
14	24.6	60	2	.66

Checking. Other conditions besides drought which cause a check in growth are known also to increase hardiness, both to drought and to cold.

Two sets of cabbage seedlings grown side by side afforded a comparison in this respect, in which permeability was also tested. One set somewhat older than the other had been severely checked in the early stages of growth by a heavy aphid infestation combined with very dull weather, and as a result showed a different morphology and much greater resistance to wilting than the younger set. The osmotic pressure of the unchecked was 10.6 atm. and of the checked 12.1 atm. The permeability of the unchecked was 0.42 and of the checked 0.86 mols per hour, a conspicuous increase with checking (Table XVI).

TABLE XVI

OSMOTIC PRESSURE AND PERMEABILITY OF CHECKED AND UNCHECKED CABBAGE SEEDLINGS (CHLORENCHYMA CELLS)

	O.P., atm.	Deplasmolysis, min. in .75 *M* thiourea	Permeability, mols per hr.
Unchecked	10.6	71	0.42
Checked	12.1	28	.86

Low nitrogen supply. Cabbage seedlings were grown in sand cultures and watered every two weeks with Knop's solution or a modification of it where low amounts of nitrogen were required. In the latter cases, calcium chloride was added to replace the calcium nitrate and potassium sulphate instead of potassium nitrate. Whether by checking growth or in some more direct fashion, low nitrogen supply increases hardiness. That it also increases permeability is shown in Table XVII.

In short, by whatever means cold resistance is induced, it is accompanied by an increase of cell permeability to polar compounds.

TABLE XVII

EFFECT OF QUANTITY OF AVAILABLE NITROGEN ON COLD RESISTANCE, OSMOTIC PRESSURE AND PERMEABILITY OF CABBAGE PLANTS

N, p.p.m.	% injury* (average of 9 plants)	O.P., atm.	Permeability,* mols per hr. (average of 5 plants)
10	25	11.8	0.55
25	15	11.2	.54
50	40	10.6	.57
100	90	10.0	.45
200	80	9.4	.32
300	85	10.6	.37

**Injury tested on one set of 9 plants.*
Permeability tested on another set of 5 plants.

Discussion

The principal conclusion from this research is that cell permeability is discovered to be correlated with hardening against frost, and indeed to show a greater and probably a more strictly parallel variation with hardiness than any of those factors to which cold resistance has hitherto been ascribed.

Hardiness and permeability vary together in the life of a plant not only in the normal seasonal rhythm and in relation to temperature change, natural or artificial, but also in response to various other factors, such as water supply, nutrition and even disease. Osmotic pressure and permeability, while frequently linked, may also vary independently, in which case frost resistance follows permeability more closely.

The degree of change in permeability (in relation to hardening) varies greatly with the type of penetrant and also with the type of plant. As regards the former, the rule seems to be that the change is greater the more polar the substance, being widest with the strong electrolyte used (potassium nitrate) and disappearing altogether with the least polar compounds (urethane, etc.). As regards differences in plant species, the permeability change depends upon their powers of cold resistance. Thus the following classification may be made, correlating hardiness and permeability.

Non-hardy—No increase in permeability on exposure to low temperature.

Semi-hardy—Permeability to water and polar non-electrolytes with small molecules (urea, etc.) increases on exposure to low temperature and to other conditions causing hardening.

Hardy—Permeability to water and polar non-electrolytes increases as in semi-hardy plants but to a much greater degree; also permeability to strong electrolytes (potassium nitrate) is measurable in the unhardened state and greatly increased on hardening.

It seems likely that the permeability test may prove to be of practical use in predicting hardiness, especially in the case of woody plants, the resistance of which is brought to the test only in exceptionally severe winters. The permeability, especially to potassium nitrate, of the cells of these plants in the winter condition, affords an easily applied measure of their potential frost resistance without the necessity of waiting for a test season. At any rate, our results demonstrate that the relative hardiness of the apple varieties tested can be judged by this means, and in breeding new types it is merely comparison within species that is required.

The degree of permeability change which our measurements record is probably unparalleled in the literature for normal non-pathological conditions. The mechanism underlying it is therefore a problem of general interest, as well as in relation to the study of hardiness. It is evident that the type of permeability which is here measured is the so-called passive or physical permeability. It obeys Fick's law of diffusion, and the temperature effect is alike on hardened and unhardened cells. The temperature coefficient is certainly high (2.1), but this applies also to other cases of passive permeability. If the permeability measured is of this type, its increase on hardening is to be ascribed to a change in the properties of the protoplasmic membrane or membranes, and, from the fact that only polar or non-lipoid-soluble substances are affected, that change is inferred to be a widening of its aqueous "pores".

As will be described in a subsequent paper, another physical change, *viz.*, a lowering of viscosity, either in the protoplasm as a whole or at least in its surface layer, also attends hardening.

Now, it is well known that salts of the alkali metals tend to produce both these effects, *viz.*, increase of permeability and liquefaction of the protoplasm. Furthermore, calcium ions which antagonize the action of these salts also tend to neutralize the effects when they result from hardening. The inference on both counts is that the underlying colloidal change associated with hardening is similar to that induced by salts of the alkalis. The nature of this change in protoplasm is suggested by physical analogy. In the case of those hydrophilic colloids which display antagonism of ions, the action of the alkalis is toward increased dispersion and hydration of the particles. This, then, seems at present the most reasonable hypothesis of the permeability increase, namely a looser packing of the organic micellae or molecules and an enlargement of the aqueous phase of the plasma membrane. The evidence is not decisive as to whether this change extends to the protoplasm as a whole. This will be discussed in a later paper.

We come now to the question of what role, if any, the permeability change plays in determining resistance to frost. The close correlation of permeability

with hardiness, when induced by the most varied factors and in most varied types of plant, inclines to the view that the correlation is more than incidental. The exact significance of the relation, however, cannot be understood unless we know precisely the mechanism of frost injury.

On the theory that over-concentration of cell solutes may be the injurious factor, escape of the more toxic ingredients of the sap might tend to obviate this (Lidforss). In spite of the high permeability of hardened cortical cells of trees to potassium nitrate as measured plasmolytically, there is no evidence of exosmosis of salts or other substance from the cells under natural conditions. Mr. J. L. Howatt, working in this laboratory, tried immersing cortical tissue of twigs in winter condition in distilled water for periods up to three days. Chemical tests applied to the extract gave only a faint unidentifiable precipitate with lead acetate. There was no evidence of appreciable leaching. It is true that in winter, substances pass from storage cells into the tracheal sap, but these are largely sugars to which we find no evidence of increased permeability as tested plasmolytically, and which are probably "secreted" by a more complex process than simple diffusion.

The very basis of the toxic concentration theory is attacked by Iljin and others on the grounds that an equal concentration of the cell sap by plasmolysis has no ill effect. While this does not eliminate the possibility that the time factor in frost action may be connected with toxic concentration, it is sufficient proof that immediate frost injury must be due to some other cause.

As will be shown in another paper, the evidence is in favor of a mechanical theory of frost injury, and certain types of mechanical injury have been observed.

Quick freezing. As regards the type of mechanical injury which results from quick freezing, the part which permeability must play is not hard to understand. The damage in this case is caused by ice forming inside the cells, and this only happens if the cell sap is cooled faster than its freezing point is lowered by concentration of its sap. The sap tends to be concentrated, of course, because crystallization always begins outside of the cells and the crystals grow by drawing water from the cells. The limiting factor is the rate at which water can escape from the cells. Hence, the higher the permeability to water, the less the danger of intracellular freezing and of death. In nature, very rapid freezing may occur momentarily after super-cooling; hence such protection is needed.

Slow freezing. There is ample evidence that on occasion damage is produced by the development of large ice masses which press upon the cells and push them apart. The smaller the crystals the less is the likelihood of mechanical injury of this type. Large crystals are the result of slow crystallization, and if the rate of crystallization is limited by the rate at which water can exosmose from the cells, a higher rate of permeability to water would tend to reduce crystal size and hence minimize mechanical damage of this

type. This may explain why large ice masses are not formed in hardy plants, and indeed it may explain *how* they are hardy. In both quick and slow freezing, the small cell size which is characteristic of hardy plants would also be useful in aiding the rapid exit of water.

Further discussion is reserved until other physical changes in the protoplasm have been described.

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ROOT ROTS OF THE RASPBERRY[1]

By G. H. Berkeley[2]

Abstract

The investigations reported here are concerned with isolations from diseased raspberry roots, preliminary inoculations with isolates, and microscopic examination of naturally and artificially infected roots.

The following fungi were isolated from naturally infected roots: *Coniothyrium Fuckelii, Cylindrocarpon radicicola, Fusarium* sp., possibly *F. orthoceras, Cylindrocladium* sp., *Pythium* spp., *Rhizoctonia Solani, Rhizoctonia* sp. (orchid type), and in preliminary inoculation experiments each fungus was found to be capable of producing necrotic lesions on healthy roots. Microscopic examinations of roots artificially inoculated with pure cultures of the above fungi, showed in the roots the presence of the fungus used for inoculation. In addition the "phycomycetous mycorrhizal" fungus already associated with root rot of strawberries and tobacco was observed to be almost always present in roots of affected raspberry plants, and, to a lesser extent, in apparently healthy roots from apparently normal plants. Nematodes, especially *Anguillulina pratensis*, the meadow nematode, were present in and on roots from certain soils, while they were absent from roots from other soils. Strawberry and raspberry seeds were sown in sterilized and non-sterilized affected soil with the result that the roots in sterilized soil appeared to be healthy, while those in the non-sterilized soil became affected with necrotic lesions.

Evidence is given which shows not only that certain symptoms of raspberry root rot are similar to the symptoms of strawberry root rot, which is considered to be a major factor in the degeneration of strawberries in both Europe and America, but also that many of the fungi and nematodes generally conceded to be associated with root rots of strawberry are likewise associated with root rots of raspberry. In the Fraser Valley, British Columbia, where certain raspberry plantations appear to be heavily infected with root rots, the possibility that these root rots may play an important role in connection with the unthriftiness of such plantations should not be overlooked.

Introduction

Macroscopic and microscopic examination of raspberry roots from certain districts in Ontario and British Columbia has shown the presence of numerous necrotic lesions on otherwise apparently healthy roots, and within such lesions, fungi and nematodes were commonly found in quantity. In Ontario the degree of infection observed to date is slight, and apparently has had little, if any, effect on growth. In raspberry material received from the Fraser Valley, British Columbia, the percentage of diseased roots was much higher. In fact in several plantations where raspberries had failed, roots were examined on which not a single healthy rootlet could be found. Though there appeared to be a direct correlation between the percentage of diseased roots and the general lack of vigor in such plants as expressed by pronounced dwarfing of canes accompanied by curling, bronzing and scorching of the leaves, and death of individual canes or the entire stool, the rotting of the roots may not be entirely responsible for the stunting or death of above-ground parts. The possibility that mineral deficiencies, unbalanced soil nutrients or toxins may predispose raspberry roots to parasitic attack must also be considered.

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Nevertheless, where a large percentage of the roots of raspberry plants are infected with parasitic fungi and nematodes, it is believed that they play an important role in this connection.

Literature

So far as the writer is aware, there is no reference in the literature to raspberries being attacked by a complex root rot, with which several different organisms may be associated. On the other hand, root rots of this nature have been reported on cereals, sugar cane, pineapples, flax, peas, tobacco, strawberries and other crops. Cereals are subject to several root rots which are generally recognized today as beng one of the major factors associated with losses in these crops. For example, browning root rot caused by *Pythium* spp. (32, 33, 34) which, according to Vanterpool and Ledingham (32) is widespread over Saskatchewan and occasions severe losses in some seasons; take-all, caused by *Ophiobolus graminis* Sacc. (10, 23, 25, 27), of which Russell (23) states that "under conditions favorable to this disease losses of 25 to 75 per cent of the crop have not been uncommon in parts of Australia, Union of South Africa, the U.S.A., and Canada"; and foot rots, caused by *Helminthosporium sativum* and *Fusarium culmorum* (6, 7, 13, 26). Pineapples in Hawaii, and sugar cane in Hawaii, the Philippines and the United States are subject to root rots caused by *Pythium* spp. (8, 9, 28). Root rots of flax (5) in the United States have been assigned to *Fusarium lini*, *Asterocystis radicis*, *Thielavia basicola*, *Pythium megalacanthum* and *Rhizoctonia* spp. Peas are affected with a serious root rot caused by *Fusarium Martii* var. *Pisi*, and *Aphanomyces euteiches*. Tobacco is subject to two distinct root rots, black root rot caused by *Thielaviopsis basicola*, and brown root rot, the cause of which is not definitely established. Recently Koch (20) has shown that several different fungi are associated with *Thielaviopsis basicola* in black root rot of tobacco. Strawberries in Europe are attacked by two types of root rot, the red core (1), and black lesion types (2). In Canada, U.S.A., Australia and Africa the black lesion type is common. Strong and Strong (29) have presented evidence to the effect that *Hainesia Lythri* (Desm.) v. Höhn., and *Coniothyrium Fuckelii* Sacc., cause a black lesion type of root rot in Michigan which is responsible for unthriftiness and patchiness in strawberry plantations, often resulting in losses ranging from 5% to as high as 60%. Berkeley and Lauder-Thomson (2) found these same two fungi and, in addition, *Fusarium orthoceras* App. and Woll., *Cylindrocarpon radicicola* Wr., and *Pachybasium candidum* Sacc., to be capable of affecting the roots of strawberry in England, thereby causing stunting and often death of affected plants. In Ontario, Walker (35) found that species of *Ramularia* were associated with root rot of strawberries. Truscott (30) has reported that species of *Pythium*, *Alternaria*, *Verticillium*, *Cylindrocladium*, *Rhizoctonia* and *Asterocystis* and a "phycomycetos mycorrhizal" fungus are also capable of parasitizing the roots of strawberry. In a microscopical study, Hildebrand (18) observed that nematodes as well as the fungi mentioned above, were generally present in the roots of

affected strawberries. Berkeley (3) describing the effects of root rot under Ontario conditions states that "individual plantations have been so attacked that what appeared to be a promising plantation in May and June was later in the season ploughed under as unprofitable". In Oregon, Zeller (36) reports a root rot of strawberry caused by *Rhizoctonia Solani* which is one of the chief factors causing variability and unevenness in Marshall strawberry plantings in the Pacific northwest.

The above citations and statements are sufficient to show that root rots are considered to be of considerable importance in the culture of various crops. Whether or not root rot will be found to be equally important in connection with raspberry culture has yet to be ascertained.

Material and Methods

The results recorded in this paper are based on (1) a small number of diseased roots observed in Ontario, and (2) root material from British Columbia collected between July and September 1935 from three farms designated for convenience as Farms P, H, and D. Also transplants were grown outdoors in pots in sterilized and non-sterilized soil from each of the three farms mentioned. In addition, strawberries and raspberries were grown from seed in sterilized and non-sterilized naturally infected soil.

Root material was prepared for microscopic examination by boiling for one minute in lacto-phenol and acid fuchsin. After boiling, the stained material was mounted in clear lacto-phenol and gently crushed under the cover slip. Inoculations were effected by the preliminary method described by Berkeley and Lauder-Thomson (2) for strawberries.

Experimental

Preliminary Tests

As a preliminary test to ascertain the possibility of parasitic attack on raspberry roots, clonal raspberry suckers growing in soil which produced normal plants were transplanted into sterilized and non-sterilized soil from Farms P, H, and D. By the end of the growing season, when some of these plants were carefully removed from the soil and washed, it was observed that the root system in the non-sterilized soil from each of the three farms was much reduced in size as compared with the roots from the same soil, sterilized (Plate II, Figs. 1 and 2). This reduction in size was largely due to the scarcity of fibrous roots. A macroscopic examination of the newer fibrous roots showed the presence of distinct necrotic lesions, especially on the roots of plants grown in non-sterilized soil. The differences in above-ground growth were equally striking, since strong, vigorous growth occurred in all sterilized soil, but exceedingly poor growth resulted in the non-sterilized soil (Plate II, Fig. 1). In this connection, it should be noted that the sterilization of normally productive soil also brought about an improvement in growth, though the difference was not so pronounced as it was in the case of the unproductive soils.

Microscopic Examination of Roots

(a) *From non-sterilized soil.* Microscopic examination of prepared rootlets showed the presence of nematodes and various fungi in the necrotic lesions. The "mycorrhizal" fungus so commonly found in strawberry roots was equally common in affected raspberry roots. In the case of plants from Farms H and D, nematodes were present in and on root lesions in great abundance, while in those from soil from Farm P, nematodes were rarely observed. *Rhizoctonia* (orchid type), *Rhizoctonia Solani*, *Pythium* sp., *Asterocystis* and unidentified mycelium were quite commonly observed in affected tissue (Plates III and IV).

(b) *From sterilized soil.* Though the fibrous roots of plants grown in sterilized soil appeared to be healthy, nevertheless the "mycorrhizal" fungus was observed to be present in roots from soils from Farms P, H and D, as also were a few nematodes in the roots from soils from Farms H and D. The explanation would appear to be that the sterilized soil had become contaminated either by means of the roots of the transplants, or because the pots were sunk in the ground outdoors. In any case, parasitic action was decidedly lessened, as indicated by the greater extent of the root system in sterilized soil, as compared to non-sterilized soil. In the case of the non-sterilized soil, it is assumed that fungus invasion had been progressing throughout the season, resulting in a greatly depleted root system, whereas in the case of the sterilized soil the roots must have been free from parasitic attack during most of the season in order to produce such a large and healthy root system. A microscopic examination of these roots supports this contention, in that fungi were comparatively scarce in the roots from sterilized soil as compared with those from non-sterilized soil; nematodes (Farms H and D) were much fewer in number, and the roots that were attacked appeared intact, whereas similar roots from non-sterilized soil were necrotic.

Seedlings Grown in Sterilized and Non-sterilized Soil

To test further the parasitic nature of this suspected root-rot complex of raspberries, seed of both raspberries and strawberries was sown in sterilized and non-sterilized soil from Farms P and H. When the seedlings showed the first true leaf they were carefully washed and examined microscopically. Distinct lesions could be seen with the naked eye on the roots of both strawberry and raspberry from non-sterilized soil, whereas no lesions were apparent on the roots from sterilized soil. When examined microscopically, the presence of the "phycomycetous mycorrhizal" fungus, *Rhizoctonia* (orchid type) and mycelium of unidentified fungi were observed in roots from the non-sterilized soil, while no fungi were observed in the roots from the sterilized soil. Also nematodes were numerous in lesions on the roots from non-sterilized soil from Farm H, though none was observed in roots from non-sterilized soil from Farm P. No nematodes were observed in roots from sterilized soil from either Farm H or Farm P.

The raspberry seedlings in the non-sterilized soil from Farms P and H were considerably stunted as compared with seedlings in the same soils sterilized.

In the non-sterilized soil from Farm H the seedlings were only $\frac{1}{4}$ inch high as compared with $\frac{3}{4}$ inch in the same soil sterilized. In the non-sterilized soil from Farm P the seedlings were $\frac{3}{4}$ to 1 inch high as compared with seedlings $1\frac{3}{4}$ to 2 inches high in the same soil sterilized. The fact that germination was low and growth poor in the soil from Farm H, even after it had been sterilized, suggests that it may be deficient in certain elements, or that it may contain a toxin or toxins.

In evaluating the above differences in growth, the beneficial effects of sterilization on the physical and chemical nature of the soil as opposed to the effect on its biology, should not be overlooked.

These tests strongly suggest a root-rot complex caused by fungi and nematodes capable of attacking either strawberries or raspberries.

Isolations from Naturally Affected Roots, Inoculations with Isolates, and Microscopic Examination of Inoculated Roots

Representatives of nine genera of fungi were isolated from lesions on diseased roots, and inoculation tests with these isolates have demonstrated that members of six genera were capable of parasitizing raspberry roots. These will be taken up separately. Microscopic examination of the inoculated roots showed the presence of the fungus used as inoculum. Also microscopic examination of naturally diseased roots showed the presence in affected root tissue of the "phycomycetous mycorrhizal" fungus and nematodes.

(a) *Coniothyrium Fuckelii* Sacc.

Inoculation with a strain of *Coniothyrium Fuckelii* Sacc., isolated from roots and crown of affected raspberry plants produced brown necrotic lesions within 4 to 5 days. Microscopic examination of such lesions showed the presence of abundant mycelium, whereas the healthy areas of roots on both sides of the lesion showed no mycelium. Check roots were healthy. The strain of *Coniothyrium Fuckelii* isolated from strawberry roots was also found to be capable of parasitizing raspberry roots. Berkeley and Lauder-Thomson (2) have shown that *Coniothyrium Fuckelii* isolated from raspberry canes was capable of attacking roots of strawberry.

(b) *Cylindrocladium* sp.

A species of this genus was isolated, which appears to be similar to *C. scoparium* Morgan, isolated from strawberries in Ontario by Truscott (30) who found it to be a virulent parasite of strawberry. Preliminary inoculation tests demonstrated that the species isolated from raspberry not only was a strong parasite on raspberry but also was equally virulent on strawberry roots. Microscopic examination of roots following artificial inoculation showed the presence of the typical mycelium and sclerotia of the *Cylindrocladium* sp., within the tissue of the roots. This evidence, therefore, suggests that *Cylindrocladium* sp., must be considered a parasite of raspberry roots (Plate III, Figs. 5 and 6).

(c) *Cylindrocarpon radicicola* Wr.

Berkeley and Lauder-Thomson (2) and Truscott (30) have demonstrated that a species of *Cylindrocarpon* is capable of parasitizing the roots of the strawberry. Van Hell (31) has proved that *C. radicicola* Wr. is capable of causing a root rot of lily and narcissus bulbs.

Three isolates of *C. radicicola* have been obtained from diseased raspberry roots, each of which has proved to be parasitic when tested. Microscopic examination of inoculated roots has shown the presence of mycelium and chlamydospores identical with those found in cultures of the isolates.

(d) *Species of Fusarium*

Several different members of this genus have been isolated, but one species which appears to be similar to *F. orthoceras* App. and Woll. as reported on strawberries by Berkeley and Lauder-Thomson (2) has been isolated on several occasions and has been proved by inoculation tests to be parasitic on raspberry rootlets. Microscopic examination of inoculated roots has shown the presence of mycelium and chlamydospores similar to those found in the culture of the fungus used as inoculum.

(e) *Species of Rhizoctonia*

Rhizoctonia spp., of both the Solani and orchid types have been isolated from affected raspberry roots. Microscopic examination of naturally affected roots has also shown the presence of these two types within the root tissue. Truscott (30), and Hildebrand (18) have recently pointed out that *Rhizoctonia* (orchid type) is found in diseased strawberry roots in Ontario, and Zeller (36) considers *Rhizoctonia Solani* Kühn, as the cause of a strawberry root rot in Oregon. Both *Rhizoctonia Solani* and a form of the orchid type, similar to, if not identical with that described by Truscott (30) and Hildebrand (18) on strawberry, have produced dead roots or distinct brown lesions on raspberry roots within 4 to 5 days after inoculation. Microscopic examination of roots artificially inoculated with *Rhizoctonia* (orchid type) has shown the presence within the affected tissue of the characteristic monilioid cells (Plate III, Fig. 3), while artificial inoculations with *R. Solani* have shown the sclerotial groups and mycelium typical of this species. Therefore as a result of this evidence these two species of *Rhizoctonia* must be considered capable of attacking the roots of raspberry.

(f) *Species of Pythium*

Members of this genus were more commonly encountered than were members of any other group. Three morphologically different strains were isolated and inoculations gave positive results with each *Pythium* isolate tested. Microscopic examinations of the artificially inoculated roots showed in each case that the typical mycelium and spores were present in the root tissue. Definite killing of the roots occurred within 3 days from time of inoculation (Plate III, Fig. 2).

(g) *The "Phycomycetous Mycorrhizal" Fungus*

An endophyte of the phycomycetous type of "mycorrhizal" fungus, very similar to the one associated with strawberry roots (18, 21, 30) is the most prominent and conspicuous organism found in raspberry roots. Though no one has as yet been successful in culturing this fungus, evidence is accumulating which points to the fact that this fungus may be a root parasite. Truscott (30) believes that this fungus is one of the more important pathogens of the strawberry and says "The evidence that this fungus is strictly a parasite when growing in the strawberry is indisputable" (Plate IV, Figs. 1-3).

Hildebrand and Koch (19) in a study of the "phycomycetous mycorrhizal" fungus in connection with tobacco and strawberry root rots, state that in some instances necrosis of tissue in tobacco roots could be correlated with the presence of the "mycorrhizal" endophyte, but in the case of strawberry roots, necrosis was seldom observed, though depletion of starch in invaded cells was a common occurrence.

In the naturally affected raspberry roots examined, the mycelium, vesicles and arbuscules of this fungus were almost always present. In some cases the root cells were so filled with this fungus that it is hard to conceive of the possibility of normal root activity under such conditions. Though the "mycorrhizal" fungus was almost invariably present in necrotic lesions of naturally affected raspberry plants, yet many apparently healthy roots contained an abundance of this fungus with no necrosis whatever. A few cases were observed where the "mycorrhizal" fungus was present in and on root hairs.

(h) *The Genus Asterocystis*

In a few diseased roots resting cells of *Asterocystis* were present in some abundance, though little or no necrosis of host tissue was observed. *Asterocystis* is generally considered to be an obligate parasite and therefore the presence of this fungus in root tissue suggests that it may be a factor in the raspberry root-rot complex (Plate III, Fig. 1).

(i) *Nematodes*

Several different nematodes have been found associated with root rots of raspberry in certain districts in British Columbia, but *Anguillulina pratensis* (de Man 1880) Goffart 1929*, was present in greatest numbers. In root material from a farm in the Hatzic area nematodes were present in great numbers both in and on root lesions. In root material from other farms nematodes were almost entirely lacking or but rarely present.

In this connection, however, it is interesting to note that no nematodes were present in roots obtained from the most severe case of degeneration, observed in British Columbia on Farm P, while nematodes, *A. pratensis* included, were unusually abundant in and on root lesions from roots obtained from plantations much less severely affected, for instance at Farm H (Plate

* *Identified by Division of Nematology, Bureau of Plant Industry, U.S. Department of Agriculture.*

III, Fig. 4). Also when both raspberry and strawberry seed was planted in the unproductive soil from the above farms and the resulting seedlings examined with the microscope as soon as the first true leaf was formed, at which time the roots were 1 to 1½ inches long, it was observed that nematodes were abundant in lesions on roots from Farm H soil, while they were lacking in lesions on roots from Farm P soil, results which are in close agreement with the findings obtained by examination of naturally infected roots from both farms.

A. pratensis is generally considered to be a root parasite capable of attacking many different plants, however, since it has not been cultured, proof of its pathogenicity on raspberry has not been possible, but the fact that *A. pratensis* in some cases was quite general in necrotic lesions on new rootlets (seedling tests) suggests parasitism, since they were invariably associated with necrotic tissue and were sometimes the first organisms observed in such lesions.

Hastings (16) considers *A. pratensis* to be the cause of root rot of narcissi and strawberries in British Columbia. The evidence submitted here points out that raspberries may also be attacked by this nematode.

Conclusions Based on Microscopic Examination and Inoculations

From the above evidence it can be confidently adduced that under certain conditions the roots of raspberries are subject to attack by the following fungi: *Coniothyrium Fuckelii, Cylindrocarpon radicicola, Cylindrocladium* sp., *Rhizoctonia Solani, Rhizoctonia* sp.,(orchid type), *Fusarium* sp. (*F. orthoceras?*), *Pythium* spp., *Asterocystis* sp., and the so-called "mycorrhizal" fungus. Also, since nematodes, especially *A. pratensis*, were unusually abundant in diseased roots from certain districts, though absent in others, *A. pratensis* must be considered as a factor of considerable importance in the root-rot complex.

Discussion

The special methods recently applied to strawberries by Berkeley and Lauder-Thomson (2), Hildebrand and Koch (19) and Truscott (30), have demonstrated that the roots of raspberries may be attacked by various fungi and nematodes resulting in lesions on roots, and rotting of root tissue. If a high percentage of the roots of a plant are thus affected, its growth of necessity must be greatly reduced, or in severe cases, the plant may be killed.

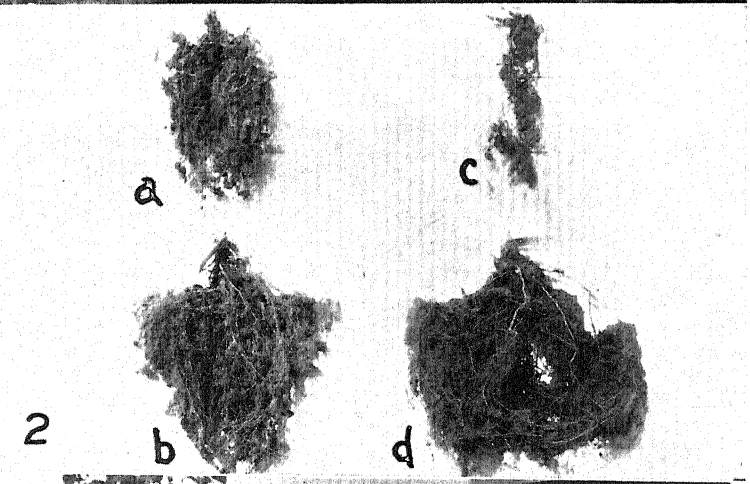
It is interesting to note that the symptoms of strawberry root rot as described by Berkeley and Lauder-Thomson (2) and Strong and Strong (29) are similar in many respects to those associated with the trouble suspected of being raspberry root rot. In both cases, affected plantations present an uneven and patchy appearance due to the small size of diseased plants and also because of the gaps caused by the death of severely affected plants (Plate I, Figs. 1 and 2). Likewise the roots of both strawberry and raspberry plants show (i) definite black lesions on the newer roots (Plate IV, Fig. 4), (ii) blackened and dead roots, and (iii) a noticeable lack of fibrous roots.

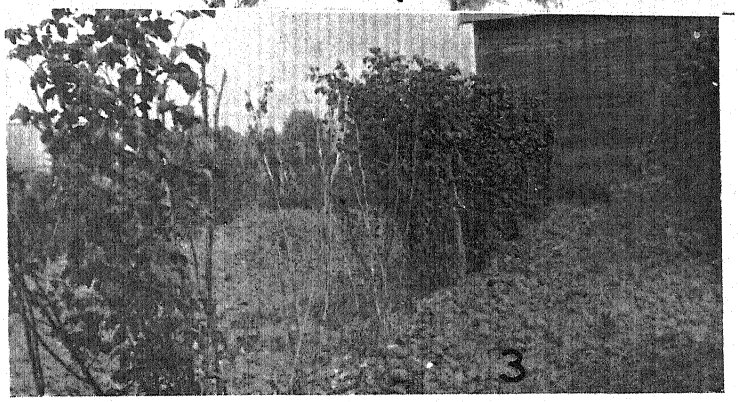
It is of particular interest that seven of eleven fungi associated with strawberry root rot are capable of parasitizing the roots of raspberry. Also microscopic examination has shown that the "phycomycetous mycorrhizal" fungus, *Asterocystis* and nematodes are present in lesions on roots of raspberries as well as in lesions on roots of strawberries affected with root rot.

Evidence has also been submitted which points out that the fungus and nematode complex is not necessarily the same in all soils, as is indicated by the absence of nematodes in one case (Farm P) and their presence in abundance in another (Farm H). Likewise *Asterocystis* was not observed in roots from some districts, while in others it was present in abundance. Moreover, it may well be that there is a seasonal variation in the same soil, as has been recorded by Berkeley and Lauder-Thomson (2) and Hildebrand (18) for strawberry root rot.

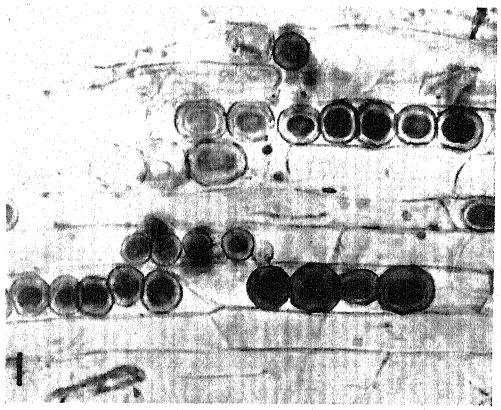
Though the evidence submitted herein indicates that raspberries, particularly in British Columbia, may be attacked by root rots, the importance of predisposing factors must not be overlooked. It is generally recognized that plants in a weakened condition from any cause are more subject to disease than plants growing under a favorable environment. For example, Vanterpool (34) has shown that browning root rot of wheat is principally confined to the wheat crop following fallow, and he concludes that "unbalanced available phosphorus and nitrate nitrogen, or perhaps in some instances low available phosphorus alone, is most likely concerned in predisposing the seedlings, or rendering them liable to fungal attack". Dickson (11) has shown that early sowings of wheat in a dry and warm soil were more severely affected with seedling blight than were later sowings in a cool and moist soil. H. Bockmann (4) reports that the blackleg form of foot rot of wheat in the Kiel district, Germany, occurs chiefly on soils of moderate or poor quality, following barley, and to a lesser extent, wheat and rye. According to Sideris and Paxton (28), excess moisture favors Pythium root rot of pineapple. Flor (14) has demonstrated that Pythium root rot of sugar cane was severe only in soils with a water content greater than 50% of the moisture-holding capacity. In Hawaii Heck (17) has demonstrated that soil fertility plays an important role in connection with root rot of sugar cane caused by *Pythium aphanidermatum*. He found that a low phosphorus and high mineral nitrogen content are features of the soils in which root rot is prevalent. Samuel (24) observed that the roots of oats growing in soil deficient in available manganese were heavily infected with an endophytic fungus, whereas roots in normal soils were not so infected. Reed and Fremont (22) in their studies with mycorrhiza of citrus trees found that "roots growing in soils that had received no fertilizers during the preceding seven years had little power to resist invasion or to digest the intracellular mycelium. The mycelium seemed to grow as a pure parasite". On the other hand "roots growing in soils which had annually received application of cover crops and stable manure appeared to develop a definite resistance to the invading fungus". Harris (15) has recently shown that many of the raspberry soils in the coastal areas of British Columbia are

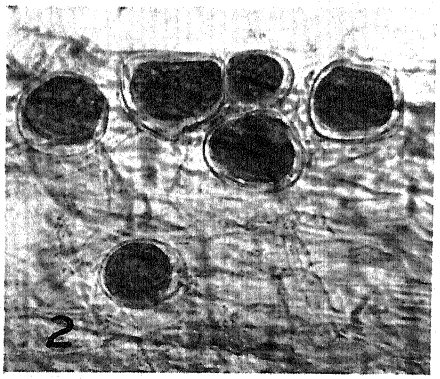
Strawberry and raspberry plantations, British Columbia. 1: Strawberry root rot in foreground. Note that many plants have died. 2: Raspberry root rot on Farm P. Note bulk of plantation has died. 3: Vigorous raspberry plants on Farm P before the "dying out" had become as severe as shown in 2.

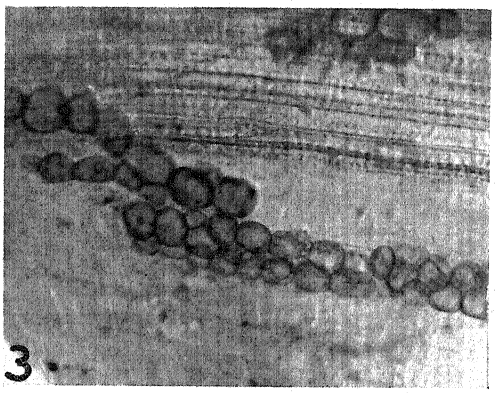


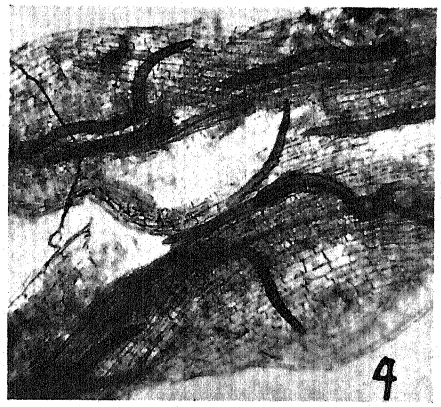


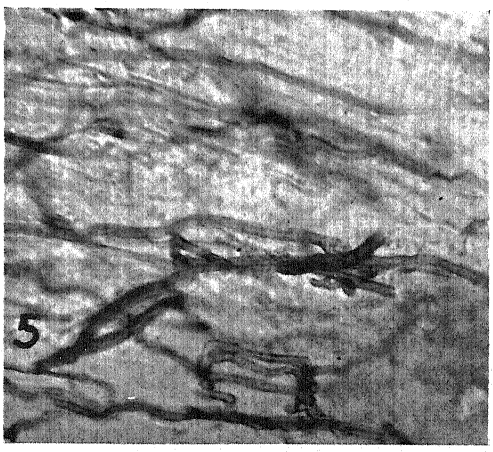
Raspberry root rot. 1: Raspberry plants grown in sterilized and non-sterilized infected soil; (a) sterilized soil from Farm H; (b) non-sterilized soil from Farm H; (c) non-sterilized soil from Farm P, and (d) sterilized soil from Farm P. 2: The roots from plants illustrated in 1: (a) roots from non-sterilized soil from Farm P; (b) roots from sterilized soil from Farm P; (c) roots from non-sterilized soil from Farm H, and (d) roots from sterilized soil from Farm H. 3: Two dead plants which had made good growth the previous year. This is the type of decline as found on Farm P.

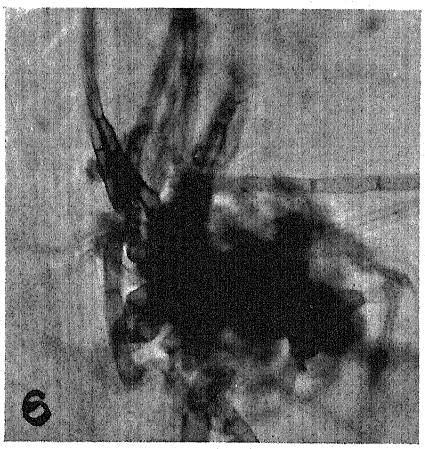




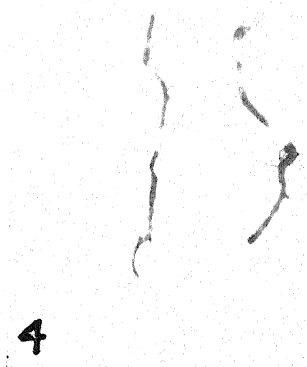


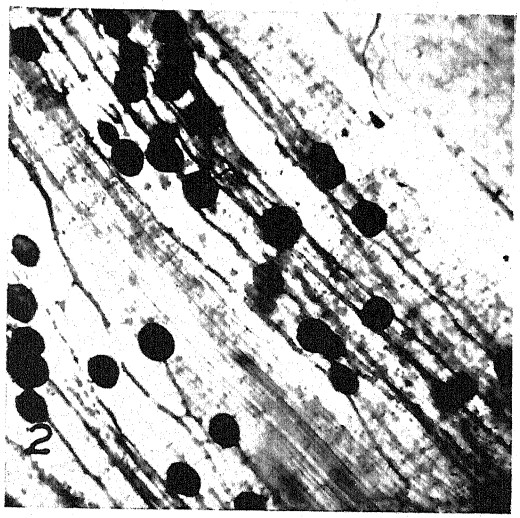






Photomicrographs. 1: Asterocystis in root tissue. 2: Spores of Pythium sp. in root tissue as result of inoculation, × 365. 3: Rhizoctonia sp. (orchid type) showing the monilioid cells in naturally affected roots, × 365. Similar cells were also observed in inoculated roots. 4: Nematodes in and on necrotic root tissue, × 365. 5: Cylindrocladium sp. Mycelium in root tissue as a result of inoculation, × 365. 6: Sclerotial-like bodies of Cylindrocladium sp., on root tissue as result of inoculation, × 365. Both the mycelium and sclerotial body were similar to those found in cultures of the inoculum.





"Phycomycetous mycorrhizal" fungus. Photomicrographs. 1: Mycelium and vesicles of "mycorrhizal" fungus, × 365. 2: Same as in 1, × 77. 3: Mycelium of "mycorrhizal" fungus. Note irregular coarse mycelium with peculiar branching and some septation. 4: Photograph of two rootlets showing necrotic lesions caused by parasitic organisms.

low in one or more of the common nutrient elements, nitrogen, phosphorus, lime and potash, a condition which would undoubtedly tend to render plants more susceptible to attack by parasitic soil fungi. It may well be, therefore, that deficiencies of soil nutrients, or unbalanced nutrients may predispose raspberries to root rot in certain districts in British Columbia.

In the above-mentioned root rots, though the importance of environment and nutrition is recognized, the diseases are nevertheless considered to be parasitic in nature. As Dickson (12) states "the parasite rightly is considered the primary cause of the parasitic disease in plants. Yet the progress of investigations is making more evident each year, the conclusion that in practically all cases the serious development of the disease is conditioned upon certain environmental factors". Again, Vanterpool (34) in his recent paper on browning root rot of wheat rightly points out that "it must not be inferred from the results of chemical soil analysis and fertilizer experiments that the disease is due merely to nutritional disturbances" since *Pythium* sp. has been demonstrated as the primary cause, though it is recognized that unbalanced nutrients may predispose the seedlings to attack.

Similarly, it has been demonstrated (this paper) that raspberry roots may be parasitized by several different soil organisms resulting in destruction of root tissue, and that therefore raspberries under certain conditions may be subject to parasitic root rots. Likewise, it is recognized that the prevalence of raspberry root rot may in no small degree depend on unbalanced nutrition, deficiencies of mineral elements, toxins or other predisposing and important conditioning factors.

Acknowledgment

The author gratefully acknowledges his indebtedness to Mr. J. J. Woods, Assistant Superintendent, Dominion Experimental Farm, Agassiz, B.C., for supplying naturally affected raspberry plants, as well as plants which had been grown at Agassiz in sterilized and non-sterilized soil from Farms P, H and D. He is likewise in debt to Mr. Woods for the photographs used in Plates I and II, and for several samples of soil in which raspberries had "failed". It would have been impossible to carry out this preliminary study on raspberry root rot without the willing co-operation so kindly extended to the author by Mr. Woods.

The author is also grateful to the Dominion Botanist who made it possible for him to visit British Columbia and make a preliminary survey of certain affected areas. In this connection, the author wishes to acknowledge his indebtedness to Messrs. J. J. Woods, H. S. McLeod, J. W. Eastham, and G. E. W. Clarke, who so kindly accompanied him during the survey and offered ready assistance and co-operation at all times.

Grateful acknowledgment is made to the Division of Nematology, Bureau of Plant Industry, U.S. Department of Agriculture, for the identification of the nematode *Anguillulina pratensis*.

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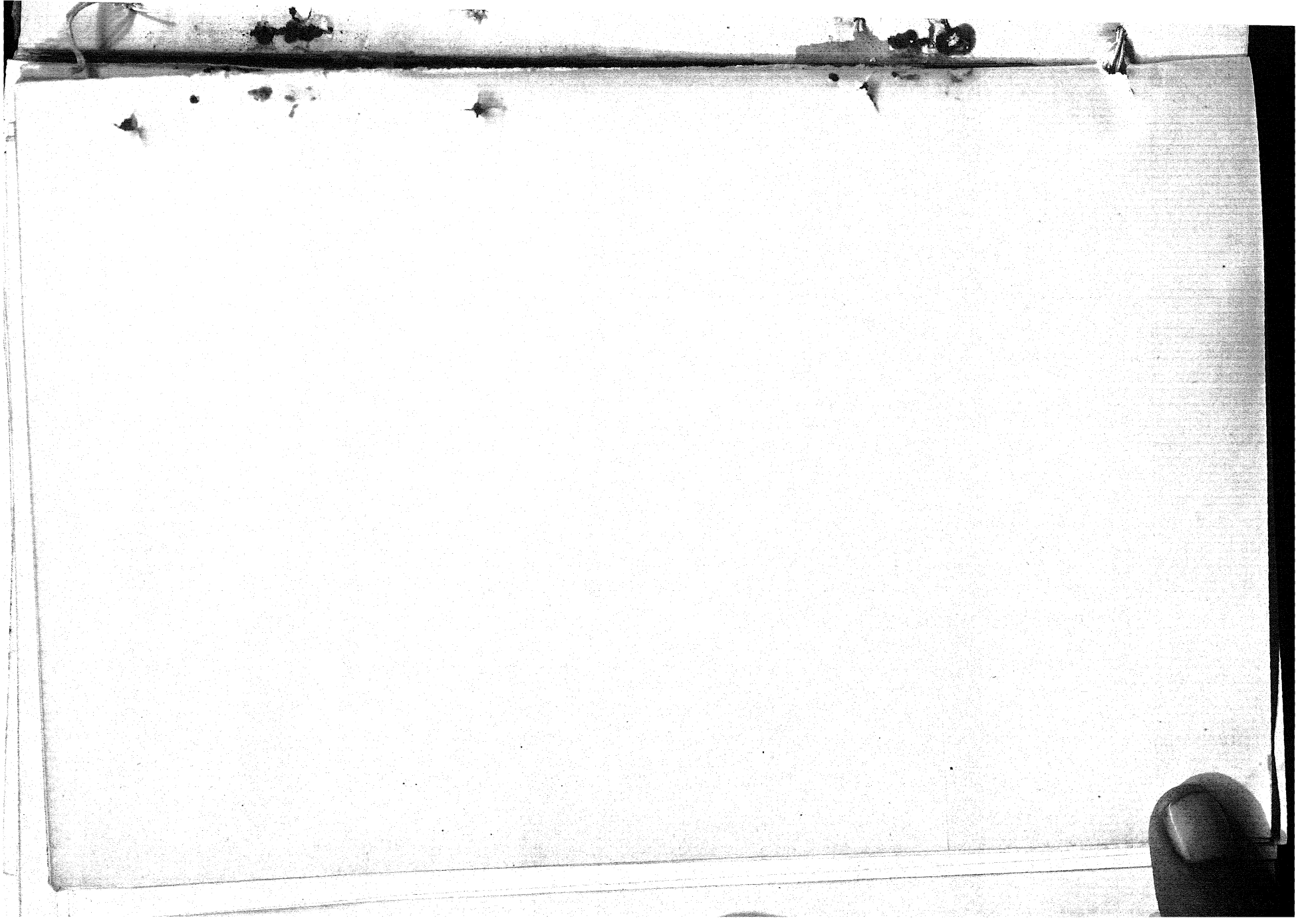

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AGRICULTURAL METEOROLOGY: SOME CHARACTERISTICS OF PRECIPITATION IN SASKATCHEWAN AND ALBERTA[1]

BY J. W. HOPKINS[2]

Abstract

A five-year moving average of autumn and winter (Sept. 1 to March 31) and spring and summer (April 1 to Aug. 31) precipitation showed undoubted variation with time over the years 1898–1934, periods of above- and below-average moisture alternating, though not with simple periodicity. There were in addition large irregular annual variations in the amounts recorded at individual stations.

In spite of irregularities, to be expected in the limited sample of years available, the frequency distribution of seasonal precipitation at Edmonton, Calgary, Battleford and Swift Current showed no gross asymmetry, seasons with precipitation in the vicinity of the average being on the whole the more numerous. There were, however, qualitative differences in the frequency distribution of the annual amounts of rain in the five individual spring and summer months. Correlation studies revealed no consistent association between the amount of precipitation in different spring and summer months of the same year, or between the totals for the autumn and winter, and for the following spring and summer period.

The average (1916–1932) percentage of days on which rain fell showed a distinct seasonal trend during spring and summer, being lowest in April and highest in June. Similar variation was noted in the average amount of rain per rainy day, which was also lowest in April and highest in June and July. In all five months the frequency distribution of the daily amounts (exclusive of zero) was decidedly skew, the smaller amounts being much the more numerous. The proportion of the total precipitation received in small daily amounts was on the average much increased during periods of subnormal rainfall.

Although there was a significant correlation between the total precipitation at different stations in the same district during the same month, analyses of variance revealed that there was also considerable local variation in the four districts studied. The monthly amounts received in daily quantities exceeding 0.30 in. per day showed relatively even greater local variation than did the total precipitation. Consequently, the intensity of precipitation, as measured by the percentage of the monthly totals accruing from the larger daily falls, was also subject to appreciable local fluctuations.

Some agricultural implications of these aspects of the weather are indicated.

Introduction

In previous agricultural meteorological investigations (5–8) the author was able to demonstrate statistically significant correlations between annual variations in rainfall and temperature, and the yield and nitrogen content of wheat crops grown in central and southern Saskatchewan and Alberta. It is now desired to present the results of a study complementary to the foregoing, dealing quantitatively with some average characteristics of the seasonal precipitation in these districts, and also analyzing the fluctuations which

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past experience has shown to occur from year to year in the precipitation at a given place, and from place to place in the same district during a given year.

This aspect of the subject was touched upon in one of the earlier papers (5), when the seasonal trend, annual variation, and intra-annual correlation of precipitation at representative meteorological stations in the districts referred to were determined for the five spring and summer months, April to August, inclusive. The present communication deals with further details of the precipitation statistics of selected stations in these districts. Specifically, consideration is given to secular variations in annual precipitation, to the frequency of occurrence of different seasonal, monthly and daily totals, to the correlation between different monthly amounts in the same season, and to the intra-monthly variability from place to place in the same district.

As in the previous studies, the precipitation data used were those compiled by the Meteorological Service of Canada (15).

Annual Variation in Monthly and Seasonal Precipitation at Specified Points

For the study of temporal variations in precipitation, the following meteorological stations were selected:

Central Alberta: Edmonton, Lacombe.
Central Saskatchewan: Battleford, Saskatoon.
Southern Alberta: Calgary, Lethbridge.
Southern Saskatchewan: Indian Head, Swift Current.

Complete records of the precipitation by months during the 37-year period 1898 to 1934 were assembled for all of these excepting Lacombe and Saskatoon, which provided slightly shorter series.

Secular Trend

Attention was first directed to the total precipitation during successive crop years. This may be conveniently divided into an autumn and winter portion (September 1 to March 31) and a spring and summer portion (April 1 to August 31). Precipitation in both of these periods fluctuated markedly from year to year at all eight stations. The situation obtaining is exemplified in Figs. 1 and 2, constructed from the observations at Calgary and Lethbridge.

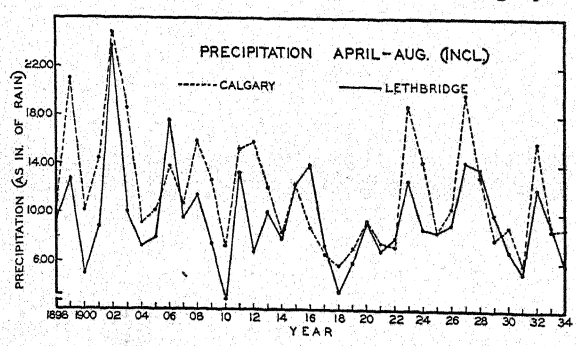

FIG. 1. *Annual variation in spring and summer (April 1 to Aug. 31) precipitation at Calgary and Lethbridge, Alberta, 1898–1934.*

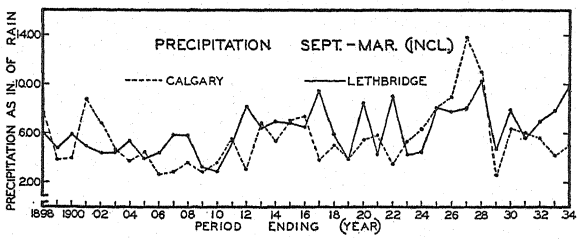

FIG. 2. *Annual variation in autumn and winter (Sept. 1 to March 31) precipitation at Calgary and Lethbridge, Alberta, 1898–1934.*

(In these and succeeding figures, precipitation, whenever occurring, is shown as "inches of rain", irrespective of whether it actually fell in the form of rain, snow or hail). As is well known, a major part of the total annual precipitation in the prairie provinces is usually received during the spring and summer months. This circumstance is clearly evident in the figures referred to; but it should also be noted that the April-August totals were correspondingly more variable than those for September-March.

Upon first examination, the annual amounts at the various stations showed little clear evidence of any definite secular trend. A dry season might be followed by another dry season, or by one of intermediate or above-average moisture. In order to bring out more clearly any temporal sequence underlying these irregular fluctuations, recourse was had to a simple process of smoothing the data for the two stations in each district. Precipitation during the same season at the two selected stations was first averaged; then the resulting seasonal means were smoothed by the calculation from them of successive values of a five-year moving average. The course of these averages is shown in Fig. 3.

Although there is still some irregular variation, the five-year averages of April-August precipitation (solid lines) show definite changes with time, now increasing to a maximum, then progressively declining until a minimum is reached, after which a further improvement takes place. Only the main features of this variation will be considered here, for in view of the pronounced local fluctuation in the incidence of precipitation, to be discussed below, observations at two stations alone cannot be expected to indicate accurately the amount received over any extensive area.

Even with this consideration in mind, it cannot be said that there is marked similarity in the course of the averages for any two of the four districts; nevertheless the movements of the smoothed values in three of them have certain features in common. On the average, summer precipitation was relatively abundant from 1900 until about 1911 at the two southern Saskatchewan stations and until 1913 at those in southern Alberta. At the central Alberta and Saskatchewan stations a dry period centred about 1907 was followed by an increase in the five-year average, which reached its maximum in 1910 and 1913, respectively. A progressive decline was then experienced in all districts, the low point of which was attained in 1916 at the southern

Saskatchewan stations, in 1917 at the central Saskatchewan ones, and not until 1919 at those in the two Alberta districts. Subsequently there was again a progressive improvement in the average, but in southern Alberta and in central and southern Saskatchewan this was once more followed by a decline, which appeared to be about its minimum at the termination of the

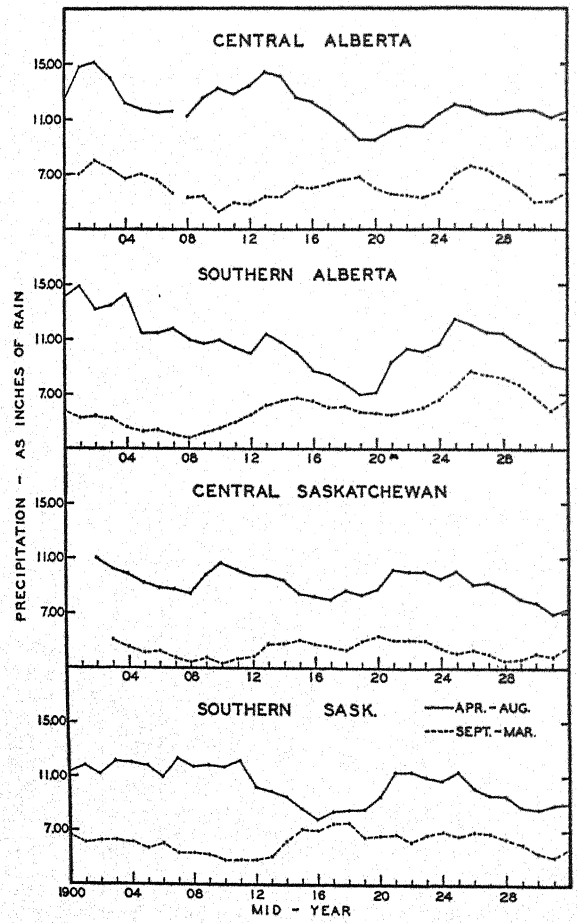

FIG. 3. *Five-year moving average of spring and summer (April 1 to Aug. 31) and autumn and winter (Sept. 1 to March 31) precipitation at meteorological stations in central and southern Saskatchewan and Alberta, 1898–1934.*

sequence. At Edmonton and Lacombe, the two central Alberta stations, this final decline did not occur, the general level of precipitation being well maintained from 1925 until 1932, the last available mid-point of the five-year average. This circumstance may lend some support to the view that although the greater part of the moisture falling in the summer rains in the prairie provinces is drawn from the Gulf of Mexico, the foothills, west-central and Peace River districts in Alberta may also receive moisture-bearing air from the northern Pacific Ocean.

It may perhaps be noted from Fig. 3 that in addition to these recurrent oscillations in the five-year average of April–August rain, there is also an apparent downward trend in the general level of the average. It seems probable, however, that this is not indicative of an actual modification of the climate in the direction of aridity, but is simply due to the fact that the commencement and termination of the series of annual observations coincided with the middle of a wet and dry period respectively. The more lengthy series of data for stations in the northern plains area of the United States, studied by Mattice (12), also showed alternating periods of above- and below-average rainfall, but after each interval of deficiency there was a succeeding recovery, with, on the whole, no long-time trend in the direction of either dryness or wetness. Nevertheless, the presence of these fluctuations necessitates caution in dealing with supposedly "normal" or average values of either rainfall or crop yields calculated over a relatively short period of years; the possibilities of bias, through the unequal incidence of wet and dry periods, being obvious.

Successive dry and moist periods at both the American and Canadian stations were far from uniform either in duration or in amplitude of deviation from the average, and cannot therefore be regarded as truly periodic. It is, of course, possible that the observed secular variation is the resultant of several periodic components, but this could only be demonstrated by means of many additional annual observations. On the other hand, a study of random-difference series by Working (19) suggests that the temporal variation in question might have arisen from the cumulative effect of combinations of independent random fluctuations from year to year in the factors controlling precipitation. Certainly these results show nothing resembling the remarkable seven-years' periodicity of total rainfall in the Sudan Gezira, to which attention is directed by Crowther and Crowther (2). From the purely meteorological point of view, this aspect of the matter would seem to be worthy of further study.

Reference has already been made to a well known feature of the "plains" type of precipitation (10, 13), namely, the occurrence of the greater part of the annual total during the summer months. Secular variation in the amount falling was greater in the summer period, but the five-year moving average of the September 1–March 31 totals at the two stations in each district also showed secular movements, which were sometimes in the same direction but sometimes in opposition to those of the April 1–August 31 amounts (Fig. 3, broken lines). On the whole, these were probably of secondary importance, agriculturally, to the fluctuations in April 1–August 31 precipitation, since they include variations in winter snowfall, which are believed (1) to have little effect on soil moisture. Furthermore, as is pointed out by Koeppe (10), the moisture content of different falls of snow, as well as the extent of drifting, may vary considerably, thus adversely affecting the comparability of the observations. The amount of autumn and spring rainfall has, however, been shown to exert a significant influence upon the following season's wheat yield (6).

It should perhaps be emphasized again that the preceding discussion of the results shown in Fig. 3 refers to smoothed values representing the average trend of precipitation. As is apparent from Figs. 1 and 2, the individual amounts recorded at any station show pronounced irregular annual fluctuations. A dry season may sometimes occur in the midst of a period of above-average moisture, and *vice-versa*. For this reason, although the onset of periods, during which precipitation will be, in general, above- or below-average, may perhaps be inferred with a moderate degree of confidence, the conditions during any specified year must remain problematical.

Frequency Distribution of Seasonal and Monthly Totals

The frequency distribution, between two-inch class intervals, of the annual totals of precipitation during the periods April 1–August 31 and September 1–March 31, 1898–1934, at four individual stations, Edmonton, Calgary, Battleford and Swift Current, is illustrated in Figs. 4 and 5.

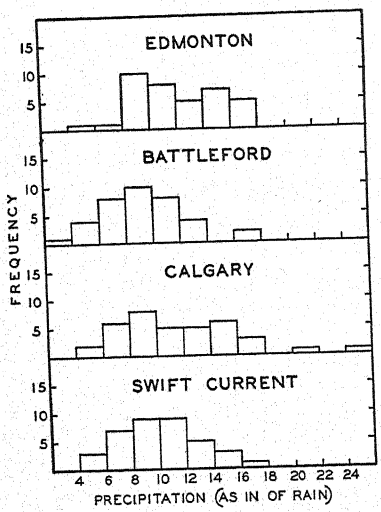

FIG. 4. *Frequency distribution of spring and summer (April 1 to Aug. 31) precipitation at meteorological stations in central and southern Saskatchewan and Alberta, 1898–1934.*

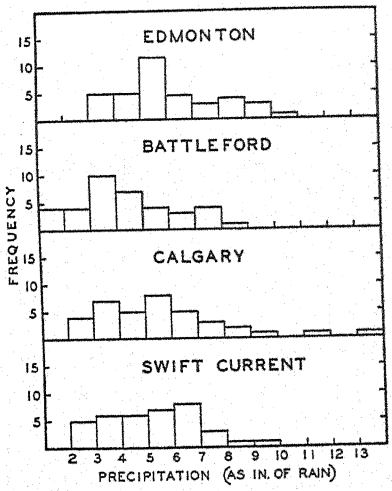

FIG. 5. *Frequency distribution of autumn and winter (Sept. 1 to March 31) precipitation at meteorological stations in central and southern Saskatchewan and Alberta, 1898–1934.*

During the period covered by these records, the general level of precipitation was clearly higher at Edmonton and Calgary than at either of the two Saskatchewan stations. The range of variation was greater at Calgary than at any of the other points, largely owing to the occurrence of a few exceptionally moist seasons. This enhanced variability is attributed to topographical effects, the proximity of Calgary to the Rocky mountain cordillera doubtless occasioning some special features in the local atmospheric circulation.

In spite of numerous irregularities, to be expected in view of the small sample of seasons available, the distributions in general incline towards symmetry, the seasonal totals for different years having tended to fluctuate around a central value. Those with precipitation in the vicinity of the average were, on the

whole, the more numerous, although in all four autumn and winter series the individual amounts were fairly evenly distributed over the range of variation.

Apart from the absence of gross asymmetry, however, little can be inferred from the available data, the number of years' observations at each station being too scanty to permit the statistical discrimination of any moderate degree of skewness. Lackey (11) has reported this to be a feature of the annual rainfall statistics for Nebraska; of the 56 years covered by observations at Lincoln, Neb., only 24 had an amount of precipitation above the average, whereas 32 were below-average. This situation seems not to prevail when the rainfall is of the monsoon type, Sankaranarayanan (17) concluding from a study of 60 or more years' records for a considerable number of stations, that there is very little justification for the assumption of non-normal annual deviations in monsoon rainfall over most of the plains of India.

Classification of the annual amounts of rain in the individual summer months April to August, inclusive, revealed interesting differences between months, not only in the average amount of precipitation received over the whole period of years, but also in its variation from year to year.

The frequency distributions for each month and station are shown in Fig. 6.

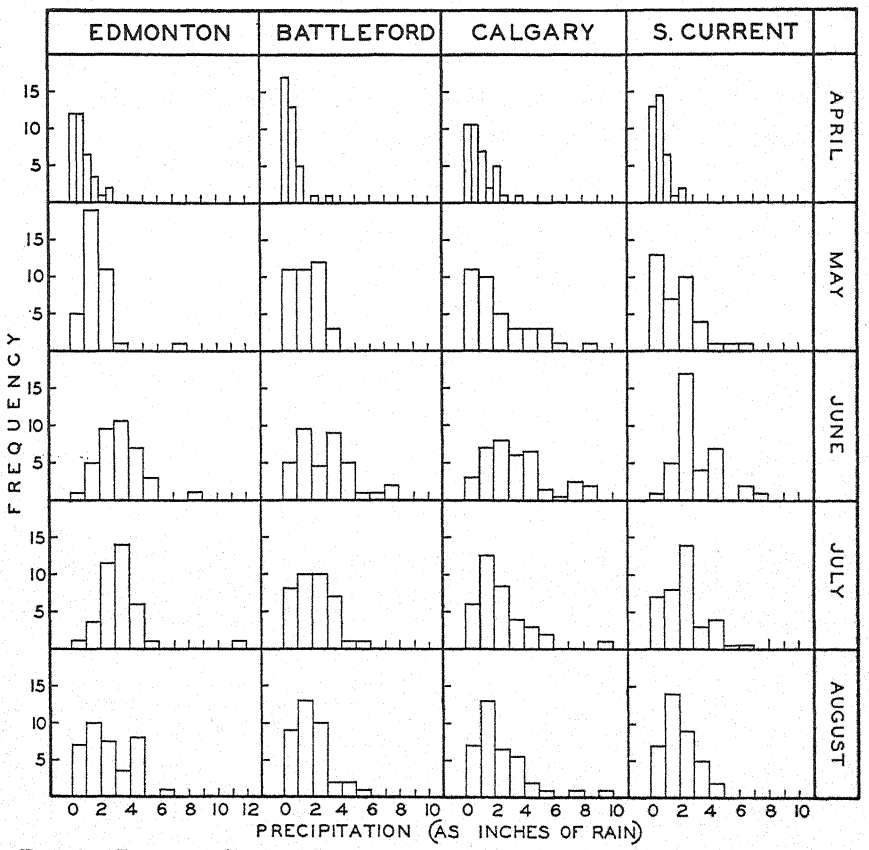

FIG. 6. *Frequency distribution of precipitation during individual spring and summer months at meteorological stations in central and southern Saskatchewan and Alberta, 1898-1934.*

At all four stations the annual distribution of April totals was markedly skew, years with the smaller amounts being the more numerous, and even moderate precipitation during this month being uncommon. In May and August there was a greater range of variation, and the skewness was less pronounced, particularly at Edmonton and Battleford, although in general below-average monthly totals were again more numerous than above-average ones. In June and July, on the average the wettest months, the total annual variation again increased, but there was a still further approach towards symmetry in the deviations, which, on the whole, tended to fluctuate about a central modal value, extreme wetness or dryness being relatively infrequent in these two months. As before, many chance discrepancies undoubtedly arose from the smallness of the number of observations available in each case.

Intra-seasonal Correlations

Association between the amount of precipitation in different periods of the same season, if present, is a climatic characteristic of obvious agricultural importance. In order to determine whether any such effects were distinguishable in the monthly and seasonal totals recorded at the preceding four representative stations, the analysis of these observations was continued by the calculation of the various correlation coefficients shown in Table I.

TABLE I

COEFFICIENTS OF CORRELATION (*r*) BETWEEN PRECIPITATION IN DIFFERENT PERIODS OF THE SAME SEASON

(37 years' data)

Periods	Edmonton	Calgary	Battleford	Swift Current
Sept.–Mar. × April–Aug.	0.14	0.23	0.13	−0.02
April × May	.01	−.21	.13	−.23
× June	−.29	−.06	−.13	−.29
× July	.04	−.11	.10	−.15
× August	.23	−.32	−.08	.17
May × June	−.10	.33	.03	.04
× July	.06	.25	.08	.27
× August	.03	.20	.36	.14
June × July	.02	.25	.12	.16
× August	.15	−.15	−.11	−.30
July × August	−.10	−.08	.11	.16

5% point = 0.33

These coefficients are all small, and only two of the 44 values exceed the 5% point (4, Sec. 34), *i.e.*, just the proportion to be expected as a result of chance combinations, in the number of seasons examined. Although at all four stations there occurred some years in which periods of above- or below-average rainfall extended into two successive summer months, it must be

concluded that these were not sufficiently frequent to be regarded as a general feature of the climate. In the long run the amounts of precipitation in the five months considered seem to have fluctuated independently from year to year. This is the more noteworthy in view of the secular variations in the five-monthly totals, discussed above. An explanation may perhaps be found in the fact that the weather of this region is predominantly influenced by cyclones (10), the behavior of each of which will be governed by a number of independently varying factors.

There was likewise no consistent relation between the total precipitation from September 1 to March 31 and that during the succeeding April 1 to August 31, from season to season.

Characteristics of Daily Precipitation

Further details of agricultural interest emerged from an examination of the records of daily precipitation during the summer months at Edmonton, Calgary, Saskatoon and Swift Current, now published by the Meteorological Service of Canada for the years 1916–1932, inclusive (15).

The pronounced seasonal march in the average amounts of rain received at these and neighbouring stations during the spring and summer months was commented upon in a previous paper (5). The 31-year average was found progressively to increase from the low value of one inch or less in April to a maximum of three inches or more in June, after which there was again a diminution in July and August, such a progression being typical of the "northern plains" type of precipitation (13). Turning now to the statistics of the number of days on which a measurable amount of rain (0.01 in. or more) fell, these also were found to exhibit a somewhat similar trend, as indicated in Table II. This variation in the number of rainy days per month was not, however, in direct proportion to that of the total rainfall, with the result that the average amount of rain per day on which rain fell also changed from month to month, attaining its maximum in June and July. The values of this average, for the four stations, are given in Table III.

TABLE II

PERCENTAGE OF DAYS ON WHICH A MEASURABLE AMOUNT OF RAIN FELL, 1916–1932

Station	April	May	June	July	August
Edmonton	26.7	31.3	43.3	42.1	42.7
Calgary	27.8	29.8	40.0	29.2	29.0
Saskatoon	21.2	27.1	38.8	31.9	29.2
Swift Current	27.8	28.5	38.4	30.0	27.5

TABLE III

AVERAGE AMOUNT OF RAIN (INCHES) PER DAY ON WHICH RAIN FELL, 1916–1932

Station	April	May	June	July	August
Edmonton	0.12	0.18	0.25	0.23	0.18
Calgary	0.15	0.17	0.26	0.26	0.21
Saskatoon	0.13	0.17	0.22	0.26	0.19
Swift Current	0.11	0.19	0.25	0.25	0.23

The moderate skewness noted above in the frequency distribution of the monthly totals becomes much more pronounced when the frequency of occurrence of different daily amounts in each month is considered. This is exemplified in Fig. 7, showing the partition of the individual daily totals, exclusive of zero, between successive class intervals of 0.10 in. In all five months at each station, the most numerous class was that comprising the smallest daily amounts, 0.10 in. and less; and the frequency of occurrence diminished progressively in the higher classes.

It was noted in a preceding paragraph that June and July, the months in which total precipitation was on the average highest, had not only the greatest number of rainy days, but also the largest average amount of precipitation per rainy day. This qualitative differentiation is further illustrated in Fig. 7, where it may be observed that these months were characterized by the occurrence of a few outstandingly large daily amounts, which materially extend the range of the frequency distribution and contribute appreciably to the increase in the average.

Supplementing this information respecting the frequency of precipitation of specified daily amounts, Fig. 8 shows the actual amount of rain contributed during the 17-year period by the daily falls of each class. Here a further sub-classification was introduced. The 17 annual totals for each month at each station were arranged in order of magnitude, and the eight driest Aprils, Mays, etc., identified. The total height of each column in Fig. 8 was then made representative of the aggregate rain received in daily amounts of the specified size during the entire 17 years, and the lower blackened part indicative of the proportion of this aggregate received during the eight driest months.

Considering first the totals for the entire 17-year period, a considerable fraction of all the rain received in April, May and August occurred in the form of daily amounts of 0.30 in. or less. In June and July such small daily amounts were offset to some extent by large rainfalls which, although occurring relatively infrequently, nevertheless accounted for an appreciable proportion of the total, particularly at the two Alberta stations, where the general level of precipitation was higher.

It may also be observed from Fig. 8 that all the months and stations included showed a qualitative differentiation between the precipitation of the whole 17 years and that during the eight driest periods, such that the latter were characterised not only by a smaller total precipitation, but also by an almost complete absence of daily amounts in excess of one inch. Consequently in such dry periods the fraction of the total precipitation accruing from small falls was correspondingly increased.

Obviously, the manner of occurrence of precipitation may be of great agricultural importance in determining the effectiveness of the total amount received. Much of the moisture falling in the form of light showers must be rapidly lost by evaporation from the surface layer of soil, never becoming available for absorption by plants. Thus in the opinion of Barnes and Hopkins (1), under western Canadian conditions rainfalls of less than 0.25 in.

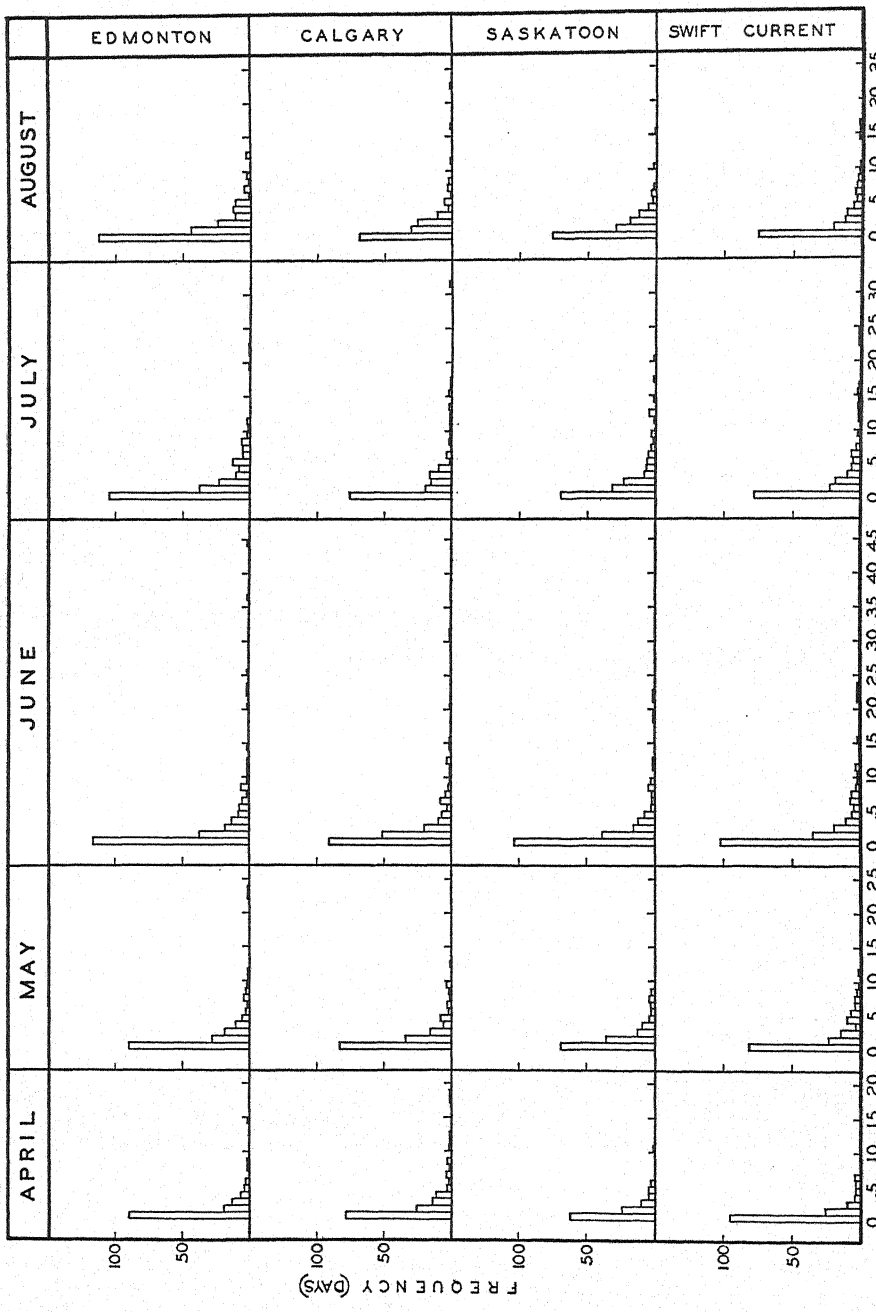

FIG. 7. *Frequency distribution of daily totals of precipitation (excluding zero) at meteorological stations in central and southern Saskatchewan and Alberta during individual spring and summer months, 1916–1932.*

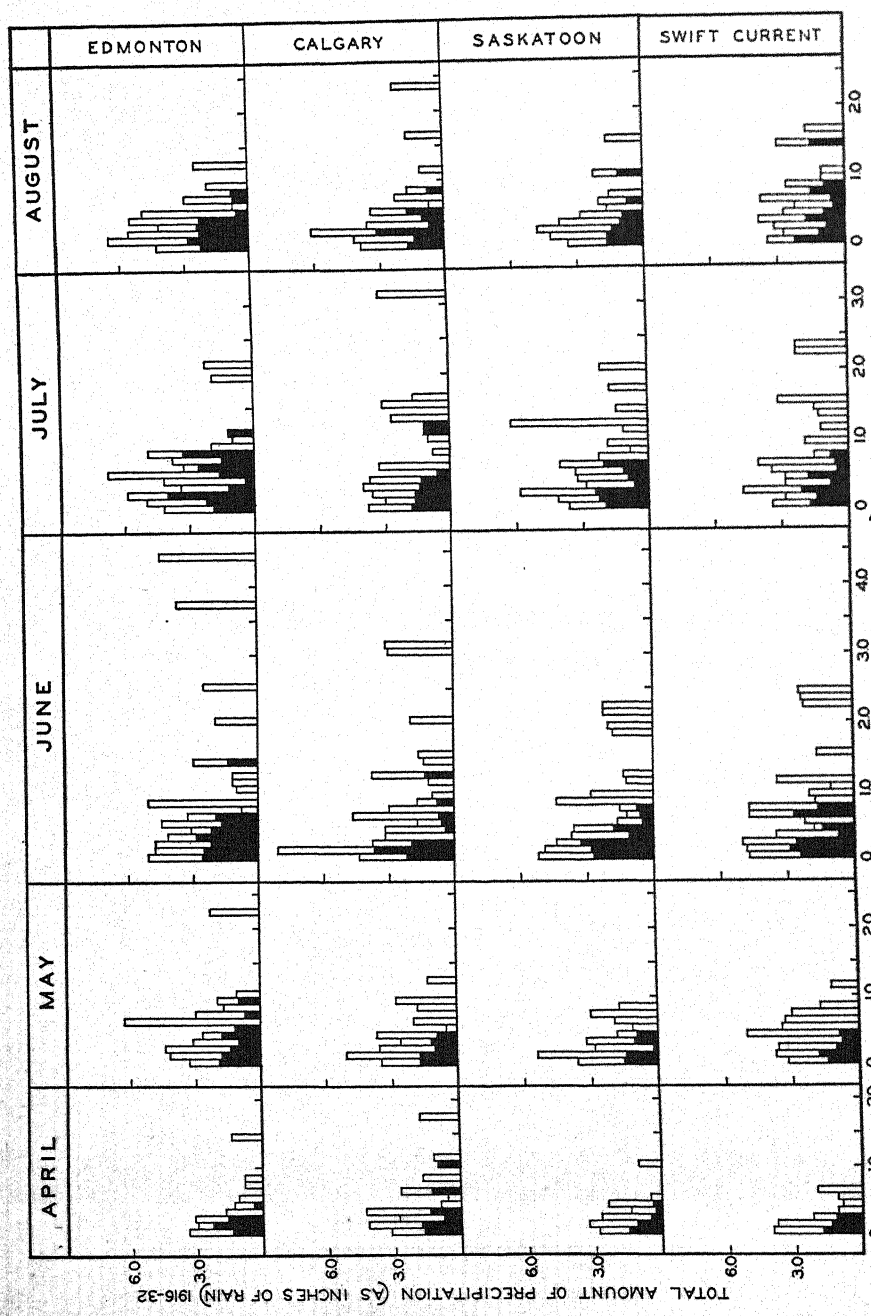

FIG. 8. *Proportion of total precipitation at meteorological stations in central and southern Saskatchewan and Alberta during individual spring and summer months, 1916–1932, received in daily amounts of specified sizes: (A) During entire period (total height of column); (B) During the eight driest Aprils, Mays, etc., at each station (lower blackened portion).*

may be of little benefit in increasing soil moisture; similarly, Daniel and Harper (3) adopted 0.50 in. per day as the lower limit of effective rainfall in Oklahoma; and McLaughlin, Blaney and Taylor (14), considering the disposition of rainfall in the Santa Ana river area of California, state that during the wet winter months "the best data available indicate that the average direct evaporation loss from the soil is about one-half acre inch per acre after each fall of rain equalling or exceeding that amount. For smaller rainfalls the entire amounts are evaporated." On the other hand, very heavy downpours lead to some loss of moisture through surface run-off, the optimum amount of unit rain consequently having an upper as well as a lower limit. In these circumstances the force of McLaughlin, Blaney and Taylor's contention (14) that "every rainfall period must be studied separately in order to arrive at the penetration for the season, since the same seasonal precipitation gives entirely different amounts of penetration under different conditions of intensity and distribution," can hardly be denied.

Table IV, obtained by summing the individual class totals represented in Fig. 8, shows the actual proportion of the total precipitation of each month at each station received in daily amounts of 0.30 in. or less, 0.31–1.50 in. and over 1.50 in., during the entire 17 years. Table V provides the corresponding figures for the eight driest Aprils, Mays, etc.

From 42 to 66% of the total April precipitation at the individual stations during the 17 years (Table IV) occurred in amounts of 0.30 in. or less per

TABLE IV

PROPORTION OF TOTAL MONTHLY PRECIPITATION OCCURRING IN VARIOUS DAILY AMOUNTS, 1916–1932

Month	Station	Statistic	Precipitation in daily amounts of		
			0.01–0.30 in.	0.31–1.50 in.	Over 1.50 in.
April	Edmonton	17-yr. av., in.	0.56	0.42	0.00
		Per cent. of total	57	43	0
	Calgary	17-yr. av., in.	0.60	0.73	0.11
		Per cent. of total	42	51	7
	Saskatoon	17-yr. av., in.	0.51	0.32	0.00
		Per cent. of total	62	38	0
	Swift Current	17-yr. av., in.	0.58	0.30	0.00
		Per cent. of total	66	34	0
May	Edmonton	17-yr. av., in.	0.70	0.91	0.14
		Per cent. of total	40	52	8
	Calgary	17-yr. av., in.	0.72	0.86	0.00
		Per cent. of total	45	55	0
	Saskatoon	17-yr. av., in.	0.69	0.74	0.00
		Per cent. of total	48	52	0
	Swift Current	17-yr. av., in.	0.62	1.08	0.00
		Per cent. of total	36	64	0

TABLE IV—*Concluded*

PROPORTION OF TOTAL MONTHLY PRECIPITATION OCCURRING IN VARIOUS DAILY AMOUNTS, 1916–1932—*Concluded*

Month	Station	Statistic	Precipitation in daily amounts of		
			0.01–0.30 in.	0.31–1.50 in.	Over 1.50 in.
June	Edmonton	17-yr. av., in.	0.87	1.61	0.75
		Per cent. of total	27	50	23
	Calgary	17-yr. av., in.	0.96	1.47	0.57
		Per cent. of total	32	49	19
	Saskatoon	17-yr. av., in.	0.85	1.25	0.48
		Per cent. of total	33	48	19
	Swift Current	17-yr. av., in.	0.85	1.47	0.51
		Per cent. of total	30	52	18
July	Edmonton	17-yr. av., in.	0.89	1.79	0.24
		Per cent. of total	31	61	8
	Calgary	17-yr. av., in.	0.61	1.08	0.47
		Per cent. of total	28	50	22
	Saskatoon	17-yr. av., in.	0.80	1.58	0.22
		Per cent. of total	31	61	8
	Swift Current	17-yr. av., in.	0.64	1.20	0.45
		Per cent. of total	28	52	20
August	Edmonton	17-yr. av., in.	0.96	1.39	0.00
		Per cent. of total	41	59	0
	Calgary	17-yr. av., in.	0.83	0.81	0.24
		Per cent. of total	44	43	13
	Saskatoon	17-yr. av., in.	0.73	0.81	0.09
		Per cent. of total	45	50	5
	Swift Current	17-yr. av., in.	0.54	1.28	0.10
		Per cent. of total	28	67	5

day, and hence may have been to a considerable extent ineffective in increasing soil moisture. (It should, however, be noted that since the rate of evaporation and transpiration from the soil depends on the evaporating power of the air, which itself changes progressively from month to month (9), the minimum effective rainfall probably also shows some seasonal trend.) In subsequent months, the average amount of rain falling on each rainy day was greater, resulting in a reduction of the average fraction of the total accounted for by daily quantities of 0.30 in. or less to 27–33% in June and 28–31% in July. At the same time, however, up to 23% of the total now fell in large amounts of over 1.50 in. per day, so that the amount received in the central and perhaps most efficient daily range rose only from 34–51% in April to 48–52% in June and 50–61% in July, although in the latter months, of course, this percentage represented a considerably greater absolute quantity of moisture.

Not all the moisture precipitated in even the largest daily falls is, of course, lost by surface run-off; indeed it seems likely that in western Canada the major part is conserved. The actual amounts thus lost will depend on the intensity of precipitation, on local topography, and on the texture and moisture-deficiency of the soil (16). According to Taggart (18), the run-off from southern Saskatchewan has been estimated to be less than 2% of the total (presumably annual) precipitation. This figure is, however, probably exceeded in districts where greater amounts and intensities of rain are experienced.

The diminution in average unit rainfall, as well as in total amount, in the eight driest instances of each month, is shown numerically in Table V. In April, from 54 to 100% of the total rain at the four stations was in daily amounts of 0.30 in. or less, and even in June small falls of this nature constituted on the 8-year average from 45 to 68% of the total. The sparse

TABLE V

PROPORTION OF TOTAL MONTHLY PRECIPITATION OCCURRING IN VARIOUS DAILY AMOUNTS DURING THE EIGHT DRIEST MONTHS, 1916–1932

Month	Station	Statistic	Precipitation in daily amounts of		
			0.01–0.30 in.	0.31–1.50 in.	Over 1.50 in.
April	Edmonton	8-yr. av., inches	0.66	0.05	0.00
		Per cent. of total	93	7	0
	Calgary	8-yr. av., inches	0.54	0.47	0.00
		Per cent. of total	54	46	0
	Saskatoon	8-yr. av., inches	0.38	0.10	0.00
		Per cent. of total	79	21	0
	Swift Current	8-yr. av., inches	0.53	0	0.00
		Per cent. of total	100	0	0
May	Edmonton	8-yr. av., inches	0.64	0.57	0.00
		Per cent. of total	53	47	0
	Calgary	8-yr. av., inches	0.54	0.26	0.00
		Per cent. of total	67	33	0
	Saskatoon	8-yr. av., inches	0.40	0.24	0.00
		Per cent. of total	62	38	0
	Swift Current	8-yr. av., inches	0.52	0.11	0.00
		Per cent. of total	82	18	0
June	Edmonton	8-yr. av., inches	0.90	1.10	0.00
		Per cent. of total	45	55	0
	Calgary	8-yr. av., inches	0.98	0.47	0.00
		Per cent. of total	68	32	0
	Saskatoon	8-yr. av., inches	1.10	0.60	0.00
		Per cent. of total	65	35	0
	Swift Current	8-yr. av., inches	1.00	0.79	0.00
		Per cent. of total	56	44	0

TABLE V—*Concluded*

PROPORTION OF TOTAL MONTHLY PRECIPITATION OCCURRING IN VARIOUS DAILY AMOUNTS DURING THE EIGHT DRIEST MONTHS, 1916–1932—*Concluded*

Month	Station	Statistic	Precipitation in daily amounts of		
			0.01–0.30 in.	0.31–1.50 in.	Over 1.50 in.
July	Edmonton	8-yr. av., inches	1.01	1.48	0.00
		Per cent. of total	41	59	0
	Calgary	8-yr. av., inches	0.63	0.64	0.00
		Per cent. of total	50	50	0
	Saskatoon	8-yr. av., inches	0.81	0.58	0.00
		Per cent. of total	58	42	0
	Swift Current	8-yr. av., inches	0.65	0.56	0.00
		Per cent. of total	54	46	0
August	Edmonton	8-yr. av., inches	0.90	0.86	0.00
		Per cent. of total	51	49	0
	Calgary	8-yr. av., inches	0.78	0.40	0.00
		Per cent. of total	66	34	0
	Saskatoon	8-yr. av., inches	0.70	0.46	0.00
		Per cent. of total	60	40	0
	Swift Current	8-yr. av., inches	0.52	0.77	0.00
		Per cent. of total	40	60	0

amount of moisture which was actually received in the dry periods was thus further reduced in average effectiveness by its disadvantageous distribution. In some instances, moreover, this situation prevailed to an extent not brought out by the average values listed in Table V, but illustrated in Fig. 9. Here, the rainfall at Calgary and Saskatoon in June of each of the 17 years, 1916–1932, is represented by a point, of which the horizontal co-ordinate specifies the monthly total and the vertical co-ordinate the proportion of this total occurring in daily quantities of 0.30 in. or less. In the moister months, this proportion was in the neighbourhood of 20%; in those of smaller total precipitation it was on the average higher, as indicated in Table V, but fluctuated considerably in different years. In several instances it attained 100%, but in others was only of the order of 40%; and in one year at Saskatoon only 10% of a June rainfall totalling but 2.04 in. occurred in amounts of 0.30 in. per day or less.

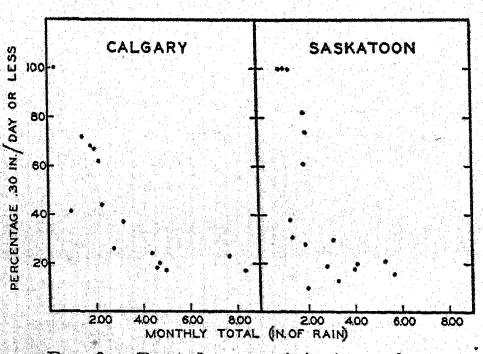

FIG. 9. *Total June precipitation and percentage of total received in daily amounts of 0.30 in. or less, at Calgary, Alberta, and Saskatoon, Saskatchewan, 1916–1932.*

This provides a further illustration of the fact that similar amounts of total precipitation may be very differently distributed in different seasons, which is of interest since it applies to periods of below-average moisture, when the penetration into the soil of the limited amounts of rain that do fall is particularly important.

Although many of the smaller daily rainfalls probably did not result in any significant increase in soil moisture, it may be presumed that the lower temperatures and increased atmospheric humidity associated with them had some effect in temporarily reducing the rate of removal of water from the soil by evaporation and transpiration. It is, therefore, desirable to know whether there was a close correlation between the total amount of rain falling in a specified month and the number of rainy days, or whether the effects of drought may be mitigated in some cases in the manner just mentioned. Fig. 10 shows the relation between total precipitation and number of rainy

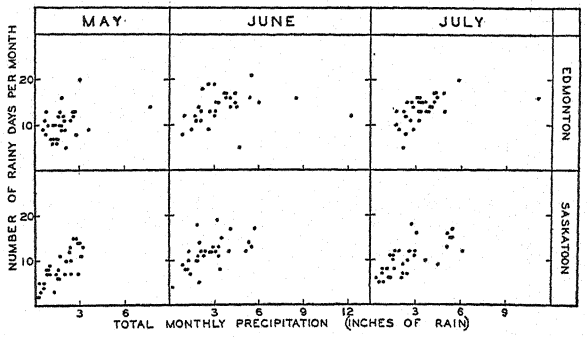

FIG. 10. *Total precipitation and number of rainy days (0.01 in. or more) per month at Edmonton, Alberta, and Saskatoon, Saskatchewan, 1900–1932.*

days per month for May, June and July, 1900–1932, at Edmonton and Saskatoon. It will be seen that in general the number of rainy days was definitely lower during the drier months, particularly in the more arid periods experienced at Saskatoon.

Local Variation in Precipitation

As is well known, the amount of precipitation during an individual rainstorm is subject to considerable local variation. In connection with the forecasting of agricultural production from weather data, the question therefore arises as to the accuracy with which observations at a limited number of meteorological stations are capable of portraying annual differences in the averages of precipitation, temperature and other components of the weather, over the considerable area (of the order of thousands of square miles) comprised by any of the Alberta and Saskatchewan crop districts.

This will, of course, depend in each case on the degree of correlation between the annual differences in the weather factor in question in individual sectors of the district. If the correlation is high, relatively few stations, situated at some distance apart, will suffice to provide a reliable indication of the

situation prevailing. Conversely, irregular or largely independent variation from year to year, for example such that some localities within the district suffered drought whilst others received at the same time an above-average amount of rain, would necessitate a larger number of stations more closely spaced.

Local Variation in Monthly Totals of Precipitation

To provide some quantitative information on this subject, the precipitation during the months of April, May, June, July and August of 10 or more separate years at a number of stations (8 to 10) in each of the four areas, central and southern Saskatchewan and Alberta, was examined in some detail. Fig. 11 shows the location of the four series of stations used

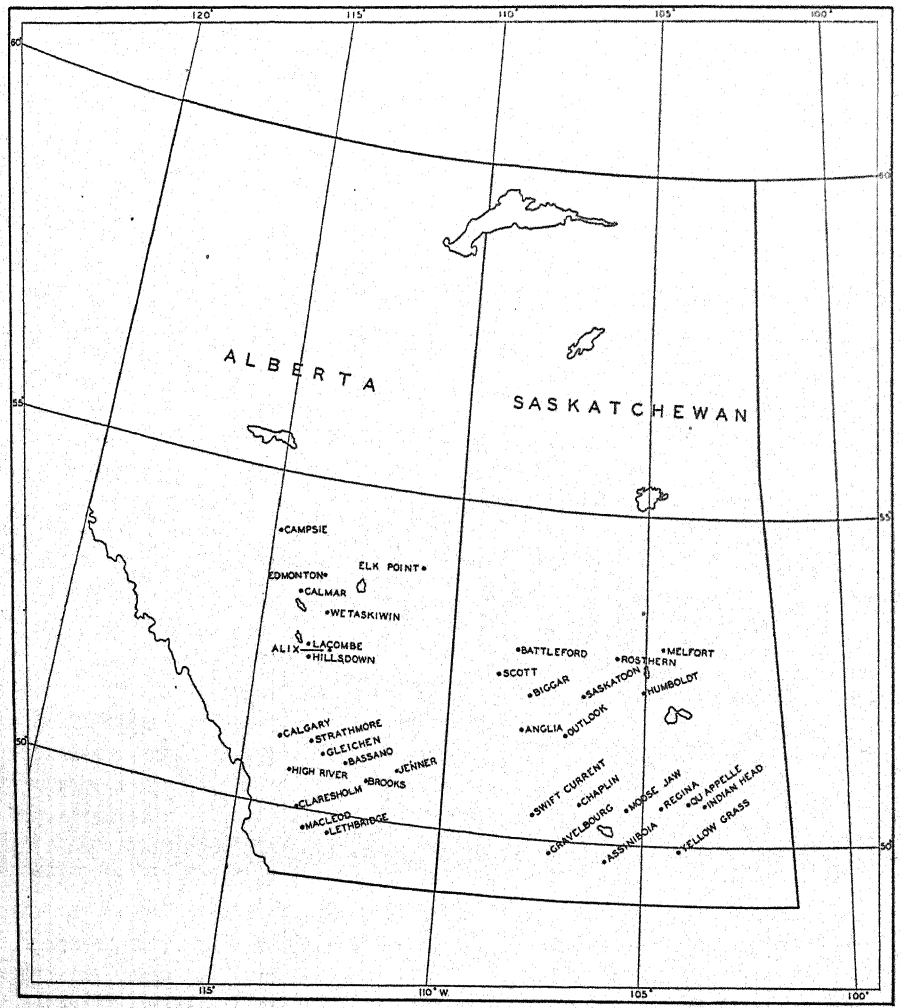

FIG. 11. *Location of meteorological stations providing data for study of local variation in precipitation.*

for this purpose; in each case a more compact grouping would have been preferable, but this could not be achieved owing to the lack of intervening stations with the required continuity of observations.

A typical example of the data thus assembled is given in Table VI, namely, total precipitation during the month of June in different years at 10 stations in southern Saskatchewan. Each row of figures in this table provides nine

TABLE VI

JUNE PRECIPITATION (INCHES OF RAIN) AT STATIONS IN SOUTHERN SASKATCHEWAN

Year	Indian Head	Moose Jaw	Qu'Appelle	Regina	Yellowgrass	Yorkton	Aneroid	Assiniboia	Chaplin	Swift Current	Mean
1923	6.99	5.03	8.22	6.09	4.83	4.90	9.33	4.18	3.93	6.82	6.03
24	2.22	1.35	2.18	1.52	2.54	1.29	4.31	2.83	4.38	2.50	2.51
25	3.47	7.11	5.30	6.29	5.88	3.59	2.24	4.15	2.93	1.56	4.25
26	1.99	2.00	2.10	1.78	4.43	1.08	4.34	5.30	.91	2.33	2.63
27	1.96	.88	1.16	2.56	1.36	3.49	2.80	1.71	1.09	1.11	1.81
28	6.05	6.07	4.68	5.56	5.18	3.37	4.63	5.53	6.11	4.80	5.20
29	1.12	1.31	1.16	1.93	1.06	.83	1.48	.67	.78	3.07	1.34
1930	2.35	2.31	4.55	2.91	3.32	3.10	1.67	2.51	2.68	4.05	2.94
31	1.18	3.95	1.54	2.48	2.00	2.35	1.65	3.23	2.15	2.45	2.30
32	3.44	5.73	4.18	3.15	4.41	3.35	2.05	4.98	4.35	2.62	3.83
Mean	3.08	3.57	3.51	3.43	3.50	2.74	3.45	3.51	2.93	3.13	3.28

degrees of freedom (4) for the estimation of the average magnitude of local fluctuations in the amount of June rain. However, the observed differences between stations may have been occasioned in part by the fact that some localities had a consistently higher or lower rainfall than others. This portion of the variance, being a factor common to all years, will not affect the accuracy with which the yearly averages indicate differences between years in the area as a whole (provided, of course, that the same series of stations is maintained throughout), and hence should be excluded from an estimate of the uncertainty attached to these averages. Following the procedure of Fisher (4, Chap. 7), the sum of the squares of the deviations of the 100 individual observations in Table VI from their general mean of 3.28 in. may in fact be divided into three additive portions, one due to annual differences in the average rainfall at all 10 stations (means of rows in Table VI), another due to differences in the average rainfall over the 10-year period at individual stations (means of columns) and a third arising from irregular variations in the individual monthly amounts after allowing for average differences between years and stations.

Such an analysis was made of each of the 20 series of observations provided by the precipitation in different years, during the five months referred to, at the specified stations in each of the four districts (2,052 individual monthly totals in all). The numerical results obtained are shown in Table VII.

The most pronounced characteristic of these is the fact that for each of the five months, in all four areas, the mean square due to variations in the

TABLE VII

ANALYSIS OF VARIANCE OF TOTAL MONTHLY RAINFALL

District	Variance due to	April		May		June		July		August	
		Deg. of freedom	Mean square	Deg. of freedom	Mean square	Deg. of freedom	Mean square	Deg. of freedom	Mean square	Deg. of freedom	Mean square
Central Alberta	Years	10	5.4827**	9	4.0725**	9	16.1850**	9	3.8883**	10	7.5863**
	Stations	7	.4158	7	1.4538**	7	1.1028	7	2.3161*	7	2.9821**
	Remainder	70	.3467	63	.4396	63	1.4975	63	1.0116	70	.7621
Southern Alberta	Years	11	6.6030**	10	23.3221**	11	22.6095**	11	6.6254**	11	8.1307**
	Stations	9	1.4231**	9	.5709	9	7.7087**	9	.8866	9	2.1091*
	Remainder	99	.5838	90	1.0130	99	1.4987	99	.7119	99	.7988
Central Saskatchewan	Years	11	1.4471**	11	8.8431**	11	16.9358**	10	16.5129**	11	6.9340**
	Stations	8	.3279	9	.0774	9	.6102	9	1.2383	8	1.4659*
	Remainder	88	.2263	99	.3973	99	1.0223	90	.9149	88	.3614
Southern Saskatchewan	Years	9	1.2178	9	13.0106	9	22.4273**	9	13.3582**	9	8.4212**
	Stations	8	.2100	8	.5862	9	.8033	9	1.7563*	9	.4500
	Remainder	72	.2053	72	.5365	81	1.5065	81	.9378	81	.3362

Mean squares due to Years and Stations which are significantly greater than the corresponding Remainder are indicated by asterisks.

**Exceeds 5% point.*

***Exceeds 1% point.*

yearly averages was very significantly greater than the mean square remainder. This indicates that there was, as might be expected, a definite correlation between the amounts of rain recorded at different stations in the same area during the same month. Some very clear examples of this may be observed in Table VI. In June, 1928, the mean precipitation at the various stations in southern Saskatchewan was decidedly above-average; and it will be seen that all 10 stations listed registered an amount considerably in excess of their respective 10-year averages. Similarly in 1929 the same stations all received below-average precipitation. These are, of course, extreme cases, but there is no doubt about the general presence of intra-monthly correlation in the precipitation at stations in the same region. It may be recollected, however, that this correlation did not extend to the amounts recorded in successive months at the same station (Table I).

Differences between stations over the 10-year period, measured by the mean squares "between stations" of Table VII, were not nearly so marked; this also might have been anticipated, in view of the absence of important topographical barriers within the districts. There were isolated instances in which some stations received significantly higher or lower average amounts of rain during certain of the five months considered than did other stations in the same district. As a rule, however, such effects, if indeed present, were not sufficiently pronounced to stand out from the remaining irregular variation.

This last, which gave rise to the mean squares or variances shown in the third, sixth, ninth and twelfth rows of Table VII, is treated in more detail in Table VIII. The first row of figures relating to each district gives the standard deviations, in inches of rain, obtained by taking the square root of the mean square remainders in Table VII, and indicates directly the uncertainty with which observations at individual stations represented variations in the amount of precipitation over the area as a whole from year to year during the months specified. The second line shows in each case this standard deviation expressed as a percentage of the corresponding mean monthly rainfall of the district, *i.e.*, the general mean of all stations over the entire period of years.

It will be seen that in addition to the intra-monthly covariation already discussed, there was an appreciable amount of irregular fluctuation in the amount of rain falling at different stations in the same district during the same month. June was the most variable month in respect of actual amount of rain, the intra-monthly, inter-station standard deviations shown in Table VIII ranging from 0.96 in. for the central Alberta stations to 1.24 in. for those in southern Saskatchewan. Assuming that the fluctuations occur with relative frequencies approximating those of a "normal" or gaussian distribution, then if observations in southern Saskatchewan were confined to a single station, the amount of June rain recorded at that station would be expected to deviate from the mean for the district as a whole by 2.03 in. or more once in 10 years on the average, and by 2.43 in. or more once in each 20 years in the long run.

TABLE VIII

STATISTICS OF LOCAL VARIATION IN TOTAL MONTHLY RAINFALL

District	Statistic	April	May	June	July	August
Central Alberta	Standard deviation (inches)	0.59	0.66	1.22	1.01	0.87
	S.D. as per cent. of district mean	50	41	42	41	35
	No. of stations required to reduce S.D. to 10%	25	16	18	16	12
	No. of stations required to reduce S.D. to 0.20 in.	9	11	37	26	19
Southern Alberta	Standard deviation (inches)	0.73	0.99	1.23	0.84	0.89
	S.D. as per cent of district mean	49	53	39	44	56
	No. of stations required to reduce S.D. to 10%	24	28	15	19	32
	No. of stations required to reduce S.D. to 0.20 in.	13	25	38	18	20
Central Saskatchewan	Standard deviation (inches)	0.48	0.63	1.01	0.94	0.60
	S.D. as per cent. of district mean	54	43	37	43	37
	No. of stations required to reduce S.D. to 10%	30	18	14	19	13
	No. of stations required to reduce S.D. to 0.20 in.	6	10	26	22	9
Southern Saskatchewan	Standard deviation (inches)	0.45	0.73	1.24	0.97	0.58
	S.D. as per cent. of district mean	56	49	38	48	36
	No. of stations required to reduce S.D. to 10%	31	24	14	23	13
	No. of stations required to reduce S.D. to 0.20 in.	5	13	38	24	8

The records for the other four months show less variation in the absolute amount of rain, but in proportion to the average monthly total, April was in fact the most variable, the intra-monthly standard deviation of the observations at individual stations in the various districts being from 49 to 56% of the mean.

Clearly, as previously suggested (6), observations at one station only in each district would be quite inadequate as an index of precipitation for crop estimating. As a matter of interest, the theoretical number of stations required to reduce the standard deviation of their mean to 10% in each case, or alternatively to a uniform level of 0.20 in. per month, was calculated and is given in the last two lines of each section of Table VIII.

Considered from the point of view of determining accurately the amount of rainfall in an agricultural district, such local variability is, of course, an unwelcome source of error. On the other hand, it may prove to be of direct advantage to the agricultural meteorologist, who seeks to ascertain by statistical methods the correlation between weather conditions and crop growth

and yield. In recently settled provinces such as Alberta and Saskatchewan, the available series of crop and weather data for periods of years at the same place are not lengthy enough to permit the study of weather effects in any detail. If, however, the weather during the same season varies appreciably from place to place within a homogeneous soil zone, and crop yields at these places are determined to a fair degree of accuracy, this intra-annual, spatial variation and co-variation, accumulated over a relatively small number of years, may make possible much more extensive correlation studies.

The individual monthly totals were also used as described below to provide some information respecting local variation in precipitation during wet and dry periods. Obviously, during a widespread drought, large differences in rainfall from point to point in the area affected are impossible. When moisture is more abundant, however, the trend of local fluctuations is less readily predictable *a priori*. It was suggested to the writer that a high average of precipitation throughout a district is most likely to result from the occurrence of several general rains, whereas, in a period of intermediate precipitation, local showers may account for a greater proportion of the total. Should this be so, local variation might in fact be greatest during such intermediate periods.

To test this point the mean square deviation between stations in each district was computed for the months of May and June of each year, and is shown plotted against the corresponding average precipitation in Figs. 12 and 13. The May results indicate definitely that the months of maximum precipitation were also those of maximum local variability. In June the situation was not so clear-cut. Some of the periods of intermediate moisture,

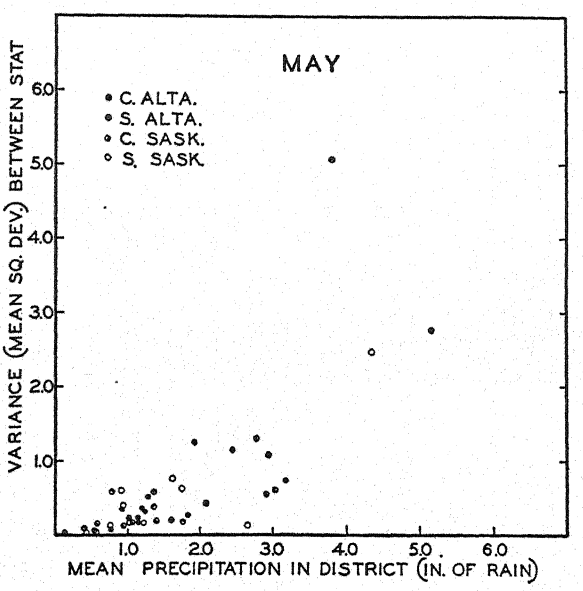

FIG. 12. *Mean precipitation and local variance by districts for the month of May, 1922–1932.*

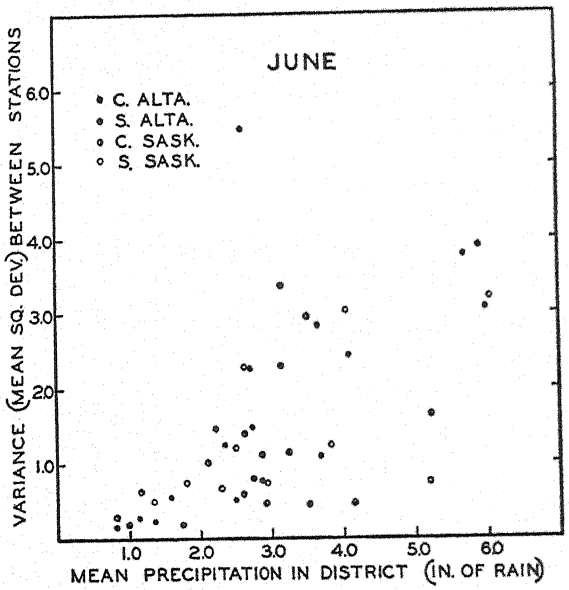

FIG. 13. *Mean precipitation and local variance by districts for the month of June, 1922–1932.*

particularly in southern Alberta, were characterized by large local variation; others, however, were below-average in this respect, and on the whole the tendency in this month also was for the amount of local variation to increase with increasing average precipitation.

Local Variation in Intensity of Precipitation

In order to ascertain whether the monthly quantities of rain received in daily amounts exceeding 0.30 in. displayed local fluctuations differing in any way from those of the total rainfall, the variance of the former quantity in each district was also examined. Owing to the labor involved in classifying the daily amounts recorded during each of the hundreds of station-months concerned, this operation was confined to the observations for May, June and July. The final results of the analysis of variance are shown in Table IX.

Generally speaking, the qualitative features of the variation are similar to those noted in the preceding discussion of total rainfall. There are still pronounced intra-monthly correlations between the amounts registered at stations in the same district, and infrequent significant differences in the 9- or 10-year average between stations. As before a considerable proportion of the total variance is attributable to irregular local differences. These were, in fact, relatively more pronounced than were the local variations in total precipitation, dealt with in Tables VII and VIII; for although rains of more than 0.30 in. per day at representative stations in each district constituted on the average only 55-70% of the total monthly precipitation (Table IV), the mean square remainders listed in Table IX are of the same order as those in Table VII. This greater relative variability of the heavier falls is shown more clearly by a comparison of the corresponding entries in Table VIII and Table X.

TABLE IX

ANALYSIS OF VARIANCE OF TOTAL MONTHLY RAIN RECEIVED IN AMOUNTS EXCEEDING 0.30 IN. PER DAY

District	Variance due to	May		June		July	
		Deg. of freedom	Mean square	Deg. of freedom	Mean square	Deg. of freedom	Mean square
Central Alberta	Years	9	1.9916**	8	14.7778**	7	5.7772**
	Stations	7	1.2344**	7	1.1057	7	1.7642
	Remainder	63	.4023	56	1.4480	49	1.0460
Southern Alberta	Years	9	17.4276**	10	12.8697**	9	2.5996**
	Stations	9	.8753	9	9.3074**	9	.6669
	Remainder	81	1.1711	90	1.7481	81	.6558
Central Saskatchewan	Years	10	5.4162**	9	8.6369**	10	10.1631**
	Stations	8	.2202	8	.5628	8	.8710
	Remainder	80	.4543	72	1.1967	80	.8160
Southern Saskatchewan	Years	9	7.5061**	9	19.6294**	9	10.3919**
	Stations	7	.8706	8	1.2604	9	1.2716
	Remainder	63	.6052	72	1.7615	81	.9924

Mean squares due to Years and Stations which are significantly greater than the corresponding Remainder are indicated by asterisks.

**Exceeds 5% point.*

***Exceeds 1% point.*

TABLE X

STATISTICS OF LOCAL VARIATION IN TOTAL MONTHLY RAIN RECEIVED IN AMOUNTS EXCEEDING 0.30 IN. PER DAY

District	Statistic	May	June	July
Central Alberta	Standard deviation (inches)	0.63	1.20	1.02
	S.D. as per cent. of district mean	66	58	61
	No. of stations required to reduce S.D. to 10%.	44	34	37
	No. of stations required to reduce S.D. to 0.20 in.	10	36	26
Southern Alberta	Standard deviation (inches)	1.08	1.32	0.81
	S.D. as per cent. of district mean	78	52	70
	No. of stations required to reduce S.D. to 10%	61	27	49
	No. of stations required to reduce S.D. to 0.20 in.	29	44	16
Central Saskatchewan	Standard deviation (inches)	0.67	1.10	0.90
	S.D. as per cent. of district mean	72	66	61
	No. of stations required to reduce S.D. to 10%	52	44	37
	No. of stations required to reduce S.D to 0.20 in.	11	30	20
Southern Saskatchewan	Standard deviation (inches)	0.77	1.33	1.00
	S.D. as per cent. of district mean	77	54	73
	No. of stations required to reduce S.D. to 10%	59	29	53
	No. of stations required to reduce S.D. to 0.20 in.	15	44	25

In view of the variation from year to year at the same station in the percentage of the total precipitation of a specified month received in daily amounts exceeding 0.30 in. illustrated in Fig. 9, it seemed desirable to examine the local fluctuations in this quantity also, thus supplementing the analysis shown in Table IX. Accordingly, the individual monthly percentages were determined and the variance of these partitioned as before into portions due to differences between years, to differences between station averages, and to the remaining irregular local fluctuations. The resulting mean squares are shown in Table XI.

TABLE XI

ANALYSIS OF VARIANCE OF PERCENTAGE OF TOTAL MONTHLY RAIN RECEIVED IN AMOUNTS EXCEEDING 0.30 IN. PER DAY

District	Variance due to	May		June		July	
		Deg. of freedom	Mean square	Deg. of freedom	Mean square	Deg. of freedom	Mean square
Central Alberta	Years	9	787.33	8	1086.23**	7	2012.94*
	Stations	7	2055.87**	7	669.80	7	940.21
	Remainder	63	529.07	56	342.70	49	671.64
Southern Alberta	Years	9	5429.18**	10	907.86*	9	1481.71
	Stations	9	789.22	9	1036.09**	9	1122.47
	Remainder	81	730.71	90	404.41	81	861.80
Central Saskatchewan	Years	10	4089.98**	9	3847.52**	10	4546.62**
	Stations	8	342.38	8	590.47	8	959.50
	Remainder	80	854.07	72	646.59	80	696.36
Southern Saskatchewan	Years	9	3636.55**	9	1662.41**	9	4243.29**
	Stations	7	1323.08	8	479.14	9	409.81
	Remainder	63	973.78	72	458.41	81	715.76

Mean squares due to Years and Stations which are significantly greater than the corresponding Remainder are indicated by asterisks.
**Exceeds 5% point.*
***Exceeds 1% point.*

Significant intra-monthly correlations in these percentages are again the main feature of Table XI, occurring in 10 of the 12 series of data examined. May precipitation at the central Alberta stations provided a marked exception to this general tendency, differences between the 10-year station averages quite overshadowing those between the 8-station monthly averages. For some reason, the percentage of the total precipitation occurring in amounts greater than 0.30 in. per day was, during the period studied, consistently greater at the western and northern stations in this district than at the southern and eastern ones. In southern Alberta in June differentiation between both years and stations was significant. The latter, however, was in this instance almost entirely attributable to the abnormally high percentages reported at High River, the 11-year average at this point being no less than 94.1%, as compared with a general average of 66.4% for the other nine stations of the group.

The intra-monthly correlations in the percentages show that in the districts concerned there were significant differences in the average intensity of precipitation during the specified months from year to year. In addition, the residual mean squares of Table XI reveal considerable irregular variation in this respect from point to point within each district during the same month. The absolute and relative standard deviations specifying the latter are given in Table XII. Of the three months studied, June, the month of maximum

TABLE XII

STATISTICS OF LOCAL VARIATION IN PERCENTAGE OF TOTAL MONTHLY RAIN RECEIVED IN AMOUNTS EXCEEDING 0.30 IN. PER DAY

District	Statistic	May	June	July
Central Alberta	Standard deviation of percentage	23	18	26
	S.D. as proportion of district mean percentage	.44	.30	.46
Southern Alberta	Standard deviation of percentage	27	20	29
	S.D. as proportion of district mean percentage	.50	.29	.51
Central Saskatchewan	Standard deviation of percentage	29	25	26
	S.D. as proportion of district mean percentage	.63	.45	.48
Southern Saskatchewan	Standard deviation of percentage	31	21	27
	S.D. as proportion of district mean percentage	.68	.34	.51

average precipitation, had the least local variation, both absolute and relative, in the percentage of the total rainfall received in daily amounts exceeding 0.30 in. The absolute standard deviation of the percentages was practically the same in all four districts for May and July, but in proportion to the mean the former month was more variable in central and southern Saskatchewan.

Qualitative variations of this nature in the incidence of precipitation may be of considerable importance in connection with the correlation between precipitation and crop yields; for, as a result of them, the effectiveness of the rainfall during the same interval in different seasons, or at different places during the same season, may be far from directly proportional to the total amounts recorded. They also have a bearing on the accuracy of determinations such as those by Yarnell (20) of rainfall intensity-frequency data for engineering purposes. Here the object was to estimate from the available observations the intensity of precipitation which may be expected to be exceeded only once in 20, 50, 100, etc., years on the average in different sections of the United States. As the number of years' observations at individual meteorological stations was limited, it was considered that a more accurate estimate of the intensity-frequency would result from pooling the records of a number of stations in the same region. Whilst this is undoubtedly so, it should not be overlooked that the gain in accuracy in each case will depend on the degree of correlation in the intensity of precipitation at the stations

thus treated. For example, if the rainfall at each of the individual stations comprising one group was totally independent, then 30 years' observations at each of five stations would provide as good a sample of the annual variation characteristic of the district as would 150 years' observations at a single station. If, however, there was intra-annual correlation in the intensity of rainfall at stations in the same district, the gain in accuracy resulting from the inclusion of additional stations would be lessened by an amount proportional to the degree of correlation. In the extreme case, in which the correlation was perfect, the inclusion of any number of stations would provide no more information concerning the variability to be expected in successive years than that yielded by the observations at a single point.

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BLACK CHAFF, A COMPOSITE DISEASE[1]

By W. A. F. Hagborg[2]

Abstract

A study was made of the etiology of wheat discolorations that resembled bacterial black chaff. Three distinct types were found. One of these was caused by *Phyt. translucens* f.sp. *undulosum*, the bacterial black chaff organism; another yielded *Alternaria* consistently on isolation; and a third, internodal melanism, appeared to be physiological in origin. *Phytomonas atrofaciens* was occasionally present in bacterial black chaff lesions.

Seed and seedling inoculation methods, unaccompanied by wounding, proved unsatisfactory for testing the pathogenicity of bacterial isolates derived from lesioned wheat plants, but inoculation accompanied by wounding of the young primary leaf, still enclosed in the coleoptile, proved to be a quick and effective method. Soil inoculation methods were ineffective. A method, involving a minimum of mechanical injury to the tissues, was developed for flooding the mesophyll of wheat leaves with a bacterial suspension.

Introduction

In the work of breeding rust-resistant varieties of wheat suitable for use in Western Canada, Goulden and Neatby (5) found that in many cases susceptibility to a melanistic disease, tentatively referred to as "black chaff", was inherited from the rust-resistant parents. The genetic linkage between rust-resistance and susceptibility to this disease appeared to be very close, although not complete. There was some doubt as to the identity of the disease, as the discolorations on some strains of wheat appeared never to produce bacterial exudate, while in the same year those on other strains of wheat produced abundant exudate. A need was felt for a better knowledge of the disease in order that the experimental error might be reduced when testing the susceptibility to it of strains and varieties of wheat. The present investigation was undertaken to gain more definite information concerning the etiology of the disease and to find a satisfactory method of reproducing it.

Historical Review of the Etiology of Black Chaff

A general review of the literature on black chaff has been made recently by Bamberg (1). The present review is concerned with only the etiology of the disease.

In 1916 Jones, Johnson, and Reddy (8) mentioned a disease on wheat very similar to the bacterial blight of barley which they (9) later described in detail.

Erwin F. Smith (12) in 1917 described a new disease of wheat that afterwards came to be known as "black chaff". He described and illustrated the field symptoms, and expressed the belief that the disease was of bacterial

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origin because of the association with it of the same bacterial organism in two successive years. In the following year, he (14) reported that the causal organism of black chaff of wheat was similar to *B. translucens* Jones, Johnson, and Reddy, and in 1919 he collaborated with Jones and Reddy (16) in describing the organism from wheat as *B. translucens* var. *undulosum*.

Jaczewski (7), in 1925, emphasized the desirability of finding the cause of pigmentation in every new variety of black pigmented wheat, because of the difficulty of distinguishing macroscopically between bacterial black chaff discoloration and natural pigmentation. This appears to be the first suggestion in the literature that pigmentation of purely physiological origin might be mistaken for bacterial black chaff.

Prissyajnyuk (11) in 1931 found bacterial black chaff in the Lower Volga region of Russia. He considered that the black pigmentation was a reaction of the plant against the organism, and suggested that possibly the same reaction could be brought about by purely external causes, such as light and temperature, independently of bacterial infection.

Two years later Broadfoot and Robertson (3) reported the occurrence in strains of Reward wheat of a type of pigmentation that appeared to be due entirely to hereditary color factors which, in a suitable environment, produced the discoloration.

Israilsky and Kazakova (6) isolated from black chaff an organism intermediate in physiology between *B. translucens* and *B. atrofaciens*, which they considered might be a variant of the former as it resembled that organism in cultural characters.

In the present year, Bamberg (1) reported the first comprehensive investigation of the disease. He found that black chaff is caused by *Bacterium translucens* var. *undulosum* and that the symptoms of the disease varied with different varieties and different environmental conditions. To the symptoms of the disease mentioned by earlier workers, he added another one, namely, discolorations on the internodes.

Nomenclature

The name "black chaff" was used by Smith (13) for the disease of wheat caused by *B. translucens* Jones, Johnson, and Reddy var. *undulosum* Smith, Jones, and Reddy, but he (15) also referred to it as "bacterial black chaff". Up to the present the former designation has been used most commonly. To the glume discoloration that they considered to be a purely physiological disease, Broadfoot and Robertson (3) applied the name "pseudo-black chaff". As will be shown later, there are at least two other diseases that fairly closely approximate in symptoms the disease originally described by Smith (12). All four of these diseases might be loosely referred to as black chaff. In order to prevent confusion, it is here proposed always to use the combination "bacterial black chaff" when referring specifically to the disease caused by *B. translucens* var. *undulosum*. Here, too, the generic name *Phytomonas* Bergey *et al* will be used. In conformity with Recommendation I of the

International Rules of Botanical Nomenclature adopted by the Fifth International Botanical Congress, 1930, the organism causing bacterial black chaff is a *forma specialis* of *Phytomonas translucens* and is therefore designated as *Phyt. translucens* f.sp. *undulosum*.

Sources of Material

Each year of the investigation (1931–1934) specimens were collected in the cereal nursery of the Dominion Rust Research Laboratory and during surveys of the agricultural areas of Manitoba. The specimens studied included all types of dark discoloration of wheat heads that could not, from macroscopic symptoms, be attributed definitely to *Septoria nodorum* Berk., *Phyt. atrofaciens* McC., or *Helminthosporium sativum* P.K. & B. In fact, the material examined included all the range of discolorations which had been identified tentatively as "black chaff" previous to the commencement of the present investigation.

Etiology

Association of Bacteria with the Diseased Condition

A microscopical examination was made to ascertain whether or not some organism was constantly associated with the diseased condition of the various tissues. Smear mounts were found most satisfactory. A small quantity of the diseased tissue was teased out in a drop of distilled water on a No. 1 cover-slip. The mount was fixed by heat and stained with Ziehl's carbol fuchsin. When the material was taken from fresh water-soaked lesions, rod-shaped bacteria of the dimensions of *Phytomonas translucens* were always present in myriads. Similarly, when the material examined was taken from dry specimens of dark linear glume lesions, such as those described and illustrated by Smith (12) for bacterial black chaff, the bacteria could always be found in large numbers; but they could not be found abundantly in other dark glume lesions of a less sharply delimited type. The latter type of discoloration was associated with sterility of the florets.

Isolation

Isolation of a causal organism from diseased specimens was attempted in as many cases as possible in order to obtain a maximum of information on the etiology of the dark discolorations. From lesions of the type that bore bacteria in large numbers, it was found possible to isolate an organism that resembled *Phyt. translucens* f.sp. *undulosum* in color and in colony type. In the present study, the organism was first isolated in 1931 from young lesions on wheat plants collected at Souris, Man. In 1932 it was obtained from two varieties of naturally infected wheat growing in the experimental plots at Winnipeg. It was quite prevalent in 1933, for it was isolated from material collected in 20 different localities. In 1934 it was found at only two points, although as thorough a survey was made in that year as in 1933. It was as prevalent in Manitoba in 1935 as in 1933.

In the earlier attempts to isolate the black chaff organism, mercuric chloride (0.1% aqueous solution) was used to surface-sterilize the diseased tissue before plating it on the surface of nutrient media. This method proved unsatisfactory, because it was found that *Phyt. translucens* f.sp. *undulosum* is very sensitive to the germicidal action of mercuric chloride. Several other methods of isolation were tried, with the result that the following one was adopted.

A small portion (about 3 sq. mm.) of the lesioned tissue was cut out with a flamed pair of scissors and dipped in 95% alcohol before being placed for 1 minute in 0.1% mercuric chloride solution. It was then washed in 10 cc. of sterile distilled water, transferred to a sterile Petri dish, and torn apart aseptically to expose the live bacteria within the tissue. Four dilution plates of beef-peptone agar were poured from the torn tissue. The plates were incubated at 26° C. for 10 days, following which they were examined with a hand lens in oblique transmitted light.

In sub-culturing from a set of isolation plates, from one to three transfers were made from colonies of the predominating type. Where two or more types occurred in abundance, additional sub-cultures were made to include these types.

In the majority of cases, the cultural characters of the isolates thus obtained conformed to those described for *Phyt. translucens* f.sp. *undulosum*.

A white bacterial organism occurred rarely in the isolation plates made from collections of black chaff. In three collections both it and the bacterial black chaff organism occurred together in the diseased tissues, as shown by the fact that they were both present in large numbers in the same dilution plates. The white organism resembled *Phytomonas atrofaciens* in cultural characters except that it did not produce a green pigment in beef-peptone agar (Difco Bacto Nutrient Agar). However, it produced a green fluorescent pigment readily in the modified Sullivan's solution recommended by Clara (4) for exhibiting green fluorescence.

Some collections failed to yield either of the above-mentioned bacterial pathogens but from them was obtained a species of *Alternaria* that resembled *A. tenuis*. From some collections no pathogen could be obtained.

All of the isolates were tested individually for pathogenicity by the inoculation of wheat seedlings.

Production of Symptoms by Artificial Inoculation

In the early stages of the investigation, no satisfactory method was known to test the pathogenicity of a large number of isolates. One isolate, which conformed to the cultural description of *Phyt. translucens* f.sp. *undulosum* and which had been found by the inoculation of sprouted seed to be pathogenic, was used in developing a method for testing the pathogenicity of the isolates. Several methods of seedling inoculation which are discussed briefly below were tried and one of these, the coleoptile-piercing method, proved to be particularly useful. It was used to test the pathogenicity of several hundred

isolates from disease lesions in order to eliminate the cultures that were non-pathogenic and to ascertain whether pathogens other than *Phyt. translucens* f.sp. *undulosum* were associated with lesions resembling those of black chaff.

It was found that the organism that was isolated repeatedly from typical bacterial black chaff produced water-soaked lesions on wheat seedlings when inoculated by this method. The organism was also found to be pathogenic in adult plants, causing lesions in leaves, peduncles, and heads. Infections were obtained ranging from a trace to a severity that caused premature death of the whole head.

The white organism, when introduced into wheat seedlings by wounding the young primary leaf with a needle dipped in inoculum, did not produce water-soaked areas, but caused the margins of the wounds to turn brown. When used to inoculate wheat heads, it produced typical basal glume rot such as is caused by *Phyt. atrofaciens.*

A mixture of spores and mycelium of the species of *Alternaria* mentioned earlier was used to inoculate heads of wheat that were in the flowering stage and were growing under greenhouse conditions in late winter at Winnipeg. The strain H-44-24 × Marquis (R.L. 590,) was used, as it had proved to be particularly susceptible under field conditions to the development of head discoloration of the type from which *Alternaria* could be isolated consistently. The inoculum was inserted in aqueous suspension between the glumes, and the plants were held in moist chambers for a period of 48 hours before being placed on a greenhouse bench. Discoloration in the empty glumes and the lemmas resulted but the controls were free from discoloration. Sterility was common in both the inoculated and uninoculated plants.

Production of Discoloration by an Unfavorable Environment

It has been mentioned above that from some collections of discolored specimens no pathogen could be isolated. In ten out of eleven collections of discolored internodes no pathogen was found, although non-pathogenic bacteria were sometimes abundant in the isolation plates. In only one of the eleven collections was a pathogen present, and it proved to be identical in cultural characters with the white bacterial organism referred to above. The fact that in ten cases out of eleven no pathogen was isolated from discolored internodes suggested the possibility that the discolored tissue was in a degenerate physiological condition and constituted a good infection court for various inocuous bacteria. Results of experiments on black chaff infection in adult plants favoured this view. In the course of such experiments it was discovered that a black discoloration, which seemed to be of non-parasitic origin, occurred in certain varieties of wheat. Plants placed in moist chambers at a high temperature for 36 hr. subsequently developed discoloration, while other plants of the same population left on the greenhouse bench developed no discoloration.

To study this type of discoloration a susceptible and a non-susceptible variety were subjected to environmental conditions conducive to the develop-

ment of the discoloration. Three lots of Pentad × Marquis wheat (R.L. 723) and three lots of Marquis were held in moist chambers at about 25° C., one lot of each variety being held for a period of 36 hr., another for 48 hr., and the third for 60 hr. The various lots were placed in the moist chambers at such times as to permit all plants to be removed from them at one time. When removed from the moist chambers, the plants were placed in a greenhouse kept at about 25° C. Twelve days later the plants were examined for darkening of the tissues. The results of the examination are shown in Table I. A large percentage of internodes, peduncles, and rachides, of R.L. 723, but not of Marquis, became discolored, but none of the glumes in either variety was affected. The younger culms were definitely less susceptible to discoloration than were the older ones.

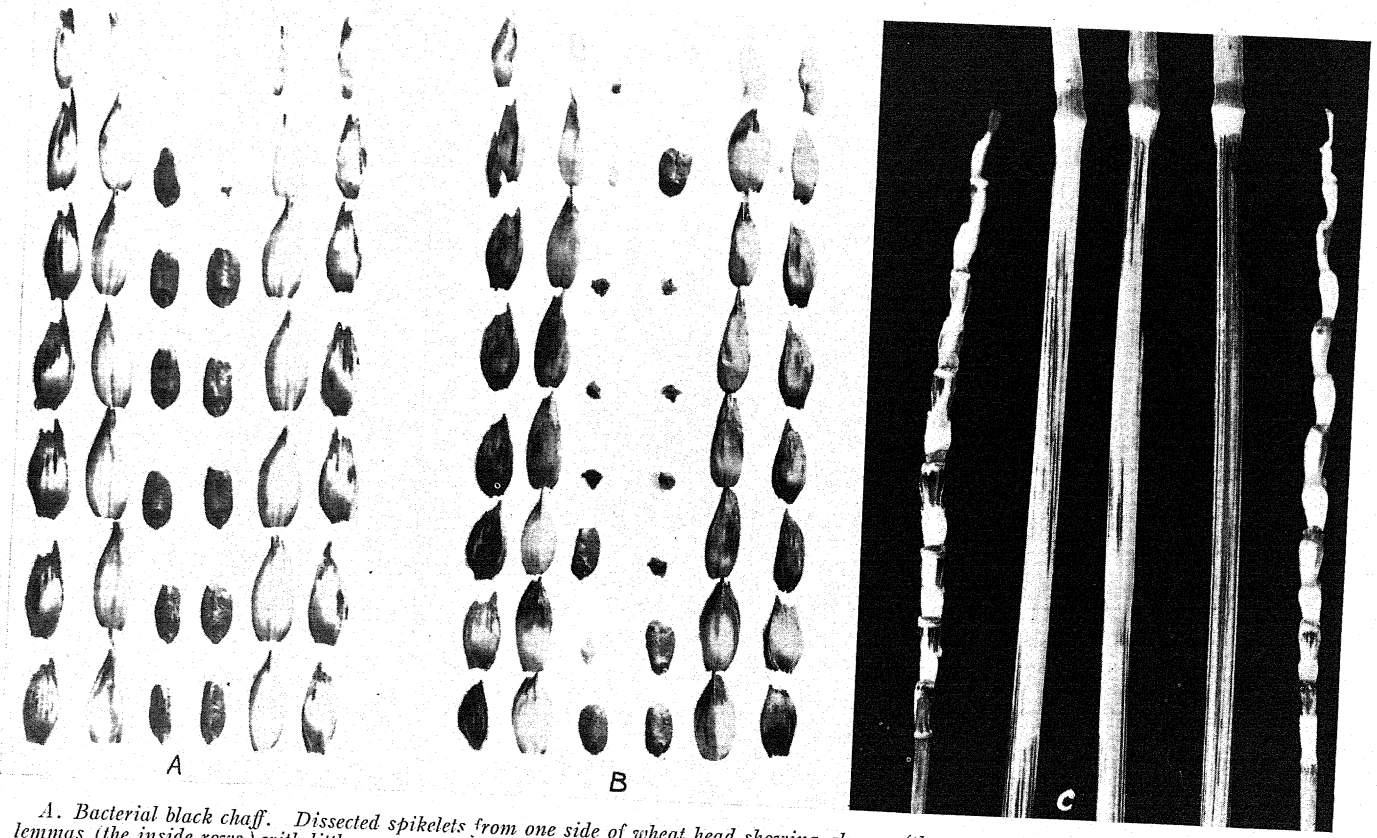
TABLE I

EFFECT OF EXPOSURE IN MOIST CHAMBERS AT 25° C. FOR VARIOUS LENGTHS OF TIME, ON THE SUBSEQUENT DEVELOPMENT OF DISCOLORATION IN TWO VARIETIES OF WHEAT

Variety	Length of exposure, hr.	Total culms	Number of discolored plant parts			
			Internodes	Peduncles	Rachides	Glumes
Marquis	36	15	0	0	0	0
	48	17	0	0	0	0
	60	14	0	0	0	0
R.L. 723	36	21	14	17	17	0
	48	17	12	11	12	0
	60	14	6	7	7	0

Isolation was attempted from six of the discolored areas, but in no case could a pathogen be recovered. It would appear that the pigmentation of the tissues was directly attributable to enclosure in the moist chambers, as no discoloration appeared on plants kept at the same temperature under normal greenhouse conditions.

This disease, which may be referred to as internodal melanism, seems to be of different genetic origin from the pseudo-black chaff described by Broadfoot and Robertson. They suggested that the symptoms were due to hereditary color factors, which, under suitable light intensity, produced dark pigmented areas on the glumes. In internodal melanism the glumes do not become discolored, while in the pseudo-black chaff the glumes are the only parts that do become pigmented. Specimens that were apparently pseudo-black chaff have been collected at Winnipeg both on Reward and on strains of hybrid origin that had Reward as one parent. Attempts to isolate an organism from pseudo-black chaff material yielded no pathogen, a result in keeping with Broadfoot and Robertson's findings. Internodal melanism appears to be distinct, too, from the definitely reddish pigmentation that sometimes occurs in the internodes, leaf-sheaths, and leaves of some varieties of wheat, such as Garnet. The pigment occurring in such reddened tissues is quite soluble



A. Bacterial black chaff. Dissected spikelets from one side of wheat head showing glumes (the two outer rows) with discoloration and lemmas (the inside rows) with little or no symptoms of the disease. The sterility of the apical florets is probably not due to bacterial black chaff. B. Discoloration by Alternaria. Dissected spikelets from one side of a wheat head showing discoloration both of glumes (the two outer rows) and lemmas (the inside rows). The cause of the pronounced sterility associated with this discoloration has not been determined. C. Internodal melanism. Rachides and stems of wheat plants showing the dark pigmentation.

PLATE I

in water, being red in acid solution and green in alkaline. It is, therefore, distinct from the pigment in internodal melanism, which is insoluble in water and in many other solvents.

Re-isolation and Cultural Comparisons

The organism that was isolated from the majority of field collections, *viz.*, *Phyt. translucens* f.sp. *undulosum*, was re-isolated repeatedly from artificially infected wheat seedlings and adult plants. The re-isolated cultures were found to produce symptoms similar to those produced by the original isolates. They were compared with the original isolates also on culture media and found to be identical with them.

Similarly, the white organism was re-isolated and compared with original isolates and found identical with them. It was also compared with isolates from field collections of what appeared to be typical basal glume rot and found similar to them in the symptoms produced following head inoculations, and in cultural characters. No green pigment was formed in beef-peptone agar by any of these cultures.

Cultures of *Alternaria* were re-isolated from the heads of wheat that became infected following artificial inoculation with the mixed suspension of Alternaria inoculum referred to above.

It can be concluded from the above studies that, while *Phyt. translucens* f.sp. *undulosum* was more or less common in Manitoba during the years of the investigation, it was not the cause of all the discolorations tentatively referred to as "black chaff" at the beginning of this investigation.

Characteristics of the Discolorations Studied

Although the study has shown that more than one cause was responsible for the discolorations, from casual inspection the discolorations appeared very similar. Close examination, however, showed definite differences between the discolorations arising from different causes. The collections from which *Phyt. translucens* f.sp. *undulosum* was isolated were characterized by a sharply delimited discoloration, chiefly confined to the chlorenchyma of the empty glumes, but sometimes also affecting the distal ends of the lemmas and the upper part of the peduncles (Plate I, A). The kernels were sometimes plump, but when the attack was heavy they were shrivelled.

In the collections from which only *Alternaria* was isolated, the margins of the discolored areas were not sharply delimited and the discoloration was diffused throughout the tissues, chiefly of the lemmas (Plate I, B). The florets were often sterile, and when sterile the ovary and stamens were frequently overgrown with mycelium.

In the type of discoloration referred to above as internodal melanism only the rachides, peduncles, and stems proper (not leaf sheaths) were affected (Plate I, C). Discoloration occurred chiefly in the chlorenchyma but vascular tissues and ground parenchyma were also sometimes black-pigmented. The margins of the discolored areas were sharply delimited. Younger culms were free from this discoloration.

Inoculation Studies of Bacterial Black Chaff

Several authors have stated that they obtained infection following artificial inoculation with the bacterial black chaff organism. Jones, Johnson, and Reddy (9), in describing the bacterial blight of barley, stated that they isolated from wheat an organism similar to the barley organism. With it they reproduced the disease on wheat by artificial inoculation. They did not describe the method. Smith (13) reported that he obtained infections on both spikelets and seedlings, but he did not say by what method of inoculation. Braun (2) obtained infection on seedlings by planting infected winter wheat and by inoculating seed with a suspension of the organism. Verwoerd (18) described a method of seedling inoculation. He sprayed seven-day-old seedlings of Hope and Kanred wheat with a heavy suspension of the organism in water. He incubated the plants at 25° C. in a moist chamber for 48 hr., and then placed them in the greenhouse, where they were kept moist by being sprayed with distilled water several times a day. This method gave results varying from "negative" to "maximum". No percentages of infection were given.

In his recent paper Bamberg (1) reported trying three different methods of inoculation *viz.*, (i) spraying with a bacterial suspension, (ii) rubbing the suspension on the leaves with the fingers and (iii) forcing the inoculum into the leaf roll of young plants or into the boot of older plants with a hypodermic syringe. He found that the third method was the most successful, the first two methods giving unsatisfactory results.

In the present investigation several methods of inoculation were tried to determine which one would give the most satisfactory results. These included different ways of inoculating seed, soil, seedlings, and mature plants with pure cultures of *Phyt. translucens* f.sp. *undulosum* isolated in this study.

(a) Seed Inoculations

When unsprouted uninjured seed was inoculated with a suspension of the organism in water, dried, and planted, a small percentage of the resulting seedlings showed infection. Sprouted seed inoculated in the same manner gave rise to a somewhat higher percentage of infected seedlings, but, when the unsprouted seed was injured by pricking the pericarp above the embryo with a sharp flamed needle before inoculation, a larger percentage of infected seedlings was obtained. Increasing the concentration of inoculum by smearing the seed with the organism before immersing it in the suspension still further

TABLE II

Summary of results of seed inoculation tests

Seed condition	State of inoculum	Location	Number of seedlings	Diseased seedlings, %
Uninjured, unsprouted	Suspension	Field	1079	13
Uninjured, unsprouted	Suspension	Greenhouse	139	14
Uninjured, sprouted	Suspension	Greenhouse	129	34
Injured, unsprouted	Suspension	Greenhouse	129	49
Injured, unsprouted	Bacterial slime	Greenhouse	45	71
Injured, unsprouted	Bacterial slime and suspension	Greenhouse	62	81

increased the percentage of infected seedlings. The results of the experiments with seed inoculation are summarized in Table II. While a fairly high percentage of infection could be obtained by inoculating injured seed, the method was tedious, and injury was accompanied by a sharp reduction in the percentage of germination. It would thus appear that seed-inoculation methods cannot be considered very satisfactory for testing the pathogenicity of cultures.

(b) Soil Inoculations

Soil inoculation was found to be ineffective both when the organism was added to sterilized soil in pots and seed planted in it, and when inoculated soil was added with the seed to the rows in a field test.

(c) Seedling Inoculations

Spraying or smearing uninjured seedlings with inoculum before placing them in moist chambers for 48 hr. resulted in a small percentage of infection, but when the leaves were injured by pricking with a needle or by rubbing with the fingers to break off trichomes, large percentages of infection resulted. The following method was found to be most satisfactory for use in testing the pathogenicity of isolates. Wheat was sown at a depth of $\frac{1}{4}$ in. in pots of loam soil. The pots were watered and set in a moist chamber until the coleoptiles reached a height of from 7 to 15 mm. above the soil. A flamed, sharp nichrome needle was dipped into the inoculum and then used to pierce the coleoptiles and enclosed primary leaves. The pots were then kept in a greenhouse at a temperature of about 25° C. Ten days after inoculation, the plants were examined for infection. This method resulted in 100% of the plants becoming infected whenever a culture of *Phyt. translucens* f.sp. *undulosum* was used as inoculum.

When the mesophyll of seedling leaves was flooded with a dilute filtered* suspension of the organism, abundant infection resulted in each of 39 leaves so inoculated. The mesophyll was flooded by means of a glass tube drawn to an outside diameter of 90–135μ and attached by means of liquid solder to a hypodermic needle fitting. The fitting was attached to a hypodermic syringe in the usual fashion. To inoculate a leaf a shallow rupture was made in the lower epidermis adjacent to the mid-vein by means of a sharp metal needle. The glass needle was inserted through the opening and forced into the mesophyll until the end of the needle was about 7 mm. from the rupture in the epidermis. Compression of the syringe flooded about a square centimeter of the mesophyll, giving it a dark green water-soaked appearance, which became normal in appearance within a few minutes. Thirty-nine seedlings were thus inoculated. After 13 days minute water-soaked points began to appear in the inoculated areas. By the fifteenth day infection was visible in every leaf that had been inoculated.

In comparison with the method in which the coleoptiles and first leaves were punctured with a needle dipped in inoculum, the injection method had a much longer incubation period. The difference, from five to eight days,

**No. 1 Whatman Filter Paper.*

was probably attributable to the greater initial amount of inoculum introduced at each inoculation point by the former method. In the needle-puncture method, a mass of bacteria were introduced into the wound, while in the mesophyll-injection method, the bacteria were introduced as a dilute filtered suspension and would therefore have to commence the attack as individuals or in very small groups. In subsequent trials of the injection method it was found that the incubation period could be reduced to as little as two days by increasing the concentration of the suspension of bacteria injected.

(d) Adult Plant Inoculations

As incubation in moist chambers at a temperature optimal for the bacterial black chaff organism resulted in abundant discoloration of the type which has been referred to above as "internodal melanism", a method of inoculation, which overcame the necessity of incubation in moist chambers, was used. Plants were inoculated by piercing their leaf sheaths with a hypodermic needle and injecting a broth culture of *Phyt. translucens* f. sp. *undulosum*. This method has been widely used for the inoculation of adult cereal plants with uredospores of stem rust since it was described by Zehner and Humphrey (19). Bamberg (1) made use of it in his recent work on black chaff.

In this experiment 19 culms of a susceptible strain of wheat were used. At the time of inoculation 2 were in head, 4 in the shot-blade stage, and 13 in the leaf-roll stage. The inoculum was developed in beef-peptone broth and injected under the leaf-sheaths by means of a sterilized hypodermic syringe fitted with a fine steel needle. After inoculation the plants were placed on a bench in the greenhouse where the temperature underwent the usual daily fluctuations.

Of the 13 infected culms 8 had infections on the glumes, 4 on the awns, 2 on the rachides, 4 on the necks, and 2 on the leaves. Some culms had infections in two or more of these parts. The infections ranged from heavy to light. No internodal infections were found.

In the younger stages of infection the lesions were water-soaked but, between the fourteenth and twenty-first day after inoculation, most of them had turned dark brown or black.

Two similar experiments, in which an agar culture of the organism suspended in sterile distilled water was used as inoculum, gave infection percentages of 57.5 and 59. This method has given moderately satisfactory results under field conditions.

Discussion

In his paper entitled, "A New Disease of Wheat", Smith (12) accurately described and illustrated the symptoms of bacterial black chaff. His photographs show well that the sharply delimited lesions occur chiefly in the chlorenchyma, thereby giving the glume a striped appearance with fusion of the stripes near its apex. He said nothing about discoloration of the internodes nor did he illustrate any stem lesions except those immediately below the head. When, however, at Winnipeg, internodal discolorations were found associated

with head symptoms of bacterial black chaff in strains of wheat of hybrid origin, it was tentatively assumed that such discolorations were additional symptoms of bacterial black chaff. From the present study it appears that this assumption was ill-founded and that the discoloration of the internodes (internodal melanism) is a physiological disorder characteristic of certain strains and varieties of wheat. In view of the regularity with which *Phyt. translucens* f.sp. *undulosum* can be isolated from bacterial black chaff lesions, failure to isolate it from any of the eleven collections of internodal melanism is good evidence that such discolorations are not caused by the bacterial black chaff organism. An extensive isolation study, to ascertain whether the organism is sometimes associated with such lesions, has been commenced.

The dark discoloration associated with bacterial black chaff is apparently not produced directly by the organism. At any rate, no black pigment is produced by *Phyt. translucens* f.sp. *undulosum* when grown in artificial culture, and, under greenhouse conditions, bacterial black chaff lesions do not turn black until several days after the maximum areas of infection have developed. Although there is no proof at present of the chemical nature of the pigment in lesions of bacterial black chaff, the similarity in color between it and the pigment in the discolored internodes suggests that the pigments are similar in nature. Besides, both pigments are highly insoluble in a large number of solvents. Stapp (17), in generalizing on the symptoms of bacterial diseases in plants, stated that increased oxidase activity sometimes ensues from disturbance in the balance of oxidizing and reducing processes in otherwise healthy cells, manifesting itself in dark discolorations. Such is probably the case in bacterial black chaff. Lewicki (10) made a detailed study of pigmentation in naturally pigmented wheats. He found that the presence of black pigmentation is due to the oxidation of catechol tannins, the reaction being catalyzed by oxidases. He considered that the pigmentation reaction gives rise to increased respiratory activity due to the great ability of the tannins to absorb oxygen. Whether or not the same chemical reactions are involved in bacterial black chaff and internodal melanism as in naturally black-chaffed wheats is not known, but it seems probable that in the former cases as in the latter the black pigmentation is due to the oxidation of a chromogen.

The relation between pseudo-black chaff and internodal melanism requires further clarification. If it is assumed that the pigment in both arises as the result of the same chemical reaction, then the failure of the glumes of R.L. 723 to develop a black pigmentation under the same conditions which resulted in black pigmentation in the internodes must have been due to improper concentration in the glumes of one or more of the reacting substances. The concentration of these substances in different plant parts very probably varies with the variety and it is conceivable that the reacting substances may be present in proper concentration only in the glumes of one variety and only in the internodes of another variety when both varieties are grown under the same conditions. The pigmentation would then manifest itself as two types which could be distinct in inheritance although the pigment was chemically

the same in both. Whether or not in pseudo-black chaff and internodal melanism the pigments are identical is not known, but the information so far available suggests that the two discolorations are distinct in inheritance. For example, some strains of Reward wheat develop pseudo-black chaff but apparently cannot be induced to develop internodal melanism, while some varieties, such as Hope, develop internodal melanism but apparently not pseudo-black chaff. On the other hand, both discolorations will develop in a pure line selection of Renown, *viz.*, R.L. 716-1, which originated in a cross between Reward and H-44-24 and therefore possibly inherited susceptibility to one of these discolorations from each parent. However, until a study is made of a family of segregates following a cross between two varieties, one of which is susceptible to pseudo-black chaff but not to internodal melanism and the other susceptible to internodal melanism but not to pseudo-black chaff, the relation between these two physiological diseases will not be well understood. Such a study should be accompanied by a detailed investigation of the influence of environmental factors on the development of pigmentation, as the possibility exists that the metabolism of even a variety such as Hope may be altered sufficiently to induce black pigment production in the glumes.

In conclusion it may be pointed out that the discovery that the discolorations formerly included under the designation "black chaff" are of multiple origin has aided an understanding of several formerly irreconcilable facts. One of these was that certain varieties in some years developed very heavy discoloration of the internodes but their glumes were quite free from discoloration, while the glumes of other varieties were heavily discolored but the internodes were relatively free from it. These reactions were difficult to understand until both internodal melanism and bacterial black chaff were known to be present.

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STUDIES ON THE INTERFERTILITY OF FOUR STRAINS OF *PUCCINIA HELIANTHI* SCHW.[1]

BY A. M. BROWN[2]

Abstract

A distinct strain of *Puccinia Helianthi* was collected on each of the following four species, *Helianthus annuus*, *H. petiolaris*, *H. tuberosus*, and *H. subtuberosus*. All possible combinations of reciprocal crosses, haploid pustule with haploid pustule, and haploid pustule with diploid pustule, were made among these four strains. Interfertile strains, when crossed, give rise to aecia in the formerly haploid pustules. On this basis, the strains fell into two groups: A, the strains on *H. annuus* and *H. petiolaris*, and B, the strains on *H. tuberosus* and *H. subtuberosus*. The two strains in each group were highly interfertile; but the two strains of one group were highly intersterile with the two strains of the other group. A parallelism exists between the crossing behavior of certain varieties of *P. graminis* and that of these two groups. It is suggested that each of these two groups of *P. Helianthi* may represent a variety.

Introduction

Studies on the crossing behavior of rust fungi have been confined to one or two cereal rusts. The present study deals with the sunflower rust (*Puccinia Helianthi* Schw.). This rust is known to be heterothallic (4) and to consist of different strains or races (2). An attempt was made to ascertain to what extent four strains of it, each collected on a different species of *Helianthus*, are interfertile.

From the studies that have been made on *Puccinia graminis* Pers., it seems that, within a variety such as *P. graminis Tritici* Erikss. & Henn., physiologic races (previously called forms) cross freely (9, 10, 11, 12, 14). The same seems to be true of races within the variety *P. rubigo-vera Tritici* Carl. (*P. triticina* Erikss.) (15). With varieties of *P. graminis* there seems to be some difference in the ease with which one variety will cross with another variety. The *Tritici* race apparently crosses rather freely with the *Secalis* race (11, 7), although the aeciospores from the *Secalis* pustules have difficulty in infecting either wheat or rye seedlings (7). Between the *Tritici* and *Agrostidis* (11, 7), and the *Tritici* and *Avenae* (8) varieties there is evidence of a rather high degree of intersterility. If degree of interfertility be taken as a criterion of the closeness of relationship between varieties, it would appear that the *Tritici* variety of *P. graminis* is more closely related to the *Secalis* variety than to either the *Agrostidis* or the *Avenae* varieties.

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Differentiation of Strains

Collections of telial material were made from *Helianthus annuus* L., *H. tuberosus* L., *H. petiolaris* Nutt., and *H. subtuberosus* Bourg.* From these collections uredial cultures were subsequently established on the respective hosts of origin. Uredospores from *H. annuus* were then used to inoculate the following species: *H. annuus*, *H. tuberosus*, *H. subtuberosus*, *H. petiolaris*, *H. subrhomboides* Rhyd., and *H. Maximiliani* Schrad. These species were likewise inoculated with uredospores from *H. tuberosus*, from *H. subtuberosus*, and from *H. petiolaris;* so that each species was inoculated with each of the four strains of rust. The results of these inoculations are given in Table I,

TABLE I

THE REACTION OF SIX SPECIES OF *Helianthus* TO COLLECTIONS OF *Puccinia Helianthi* OBTAINED FROM FOUR DIFFERENT SOURCES

Source of rust	*H. annuus*	*H. tuberosus*	*H. subtuberosus*	*H. petiolaris*	*H. subrhomboides*	*H. Maximiliani*
H. annuus (Mammoth Russian)	S	O	O	O	O	O
H. tuberosus	S*	S	S	O	S*	O
H. subtuberosus	R	O	S	O	O	O
H. petiolaris	S	O	R	S	O	O

* *Uredia on upper leaf surface only.*
S = *susceptible;* R = *resistant;* O = *immune.*

and they indicate that a distinct strain of rust was present on each of the four species, *H. tuberosus*, *H. annuus*, *H. subtuberosus*, and *H. petiolaris*. The host reactions were generally clearly defined, and no difficulty was experienced in distinguishing between strains. Plate I, A and B, shows the reaction of *H. annuus* to two of the strains.

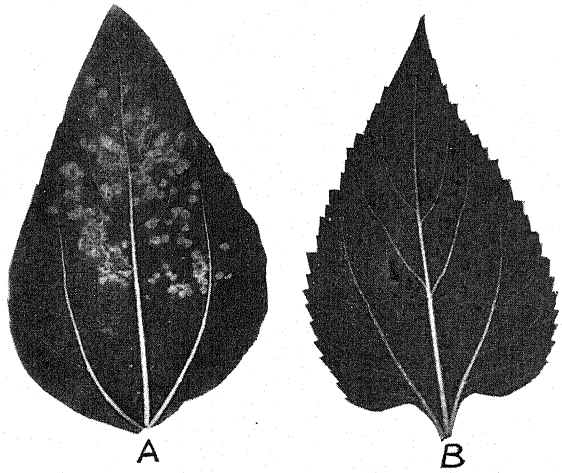
Crossing Experiments

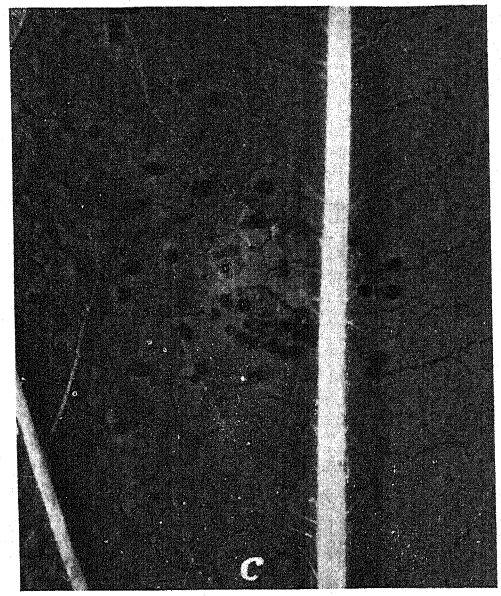
A. By the Transfer of Pycnial Nectar

Telial material of each strain was produced in the greenhouse, and from this material haploid pustules of each strain were obtained on plants of Mammoth Russian sunflower, each plant being infected by a single strain. These pustules were made use of in the crossing experiments described below. The plants were protected from insects by screen-wire cages. To ensure reasonable certainty that the pustules used in the crosses were really haploid, only isolated pustules that were still haploid at the end of 21 days were chosen for the experiments.

* *Acknowledgment is made to Dr. R. C. Russell, Dominion Laboratory of Plant Pathology, Saskatoon, Sask., for providing material of H. petiolaris and H. subtuberosus.*

PLATE I





A and B. Reactions of H. annuus to two strains of P. Helianthi. A. resistant reaction to the H. subtuberosus strain; B. susceptible reaction to the H. annuus strain. ×¼. C. Diploid mycelia of the annuus strain of P. Helianthi in contact with a haploid mycelium of the tuberosus strain. No aecia are produced.

Following the method developed by Craigie (5), reciprocal crosses were made between all possible pairs of these four strains of rust. Briefly, the method was as follows. Pycnial nectar was collected from a number of haploid pustules of one strain to make a composite sample of nectar, and similar samples were collected from haploid pustules of each of the three strains. In making a cross between two strains, composite nectar of one strain was applied to the upper surface of pustules of the other strain, and nectar of the latter strain to pustules of the former. If, after a reciprocal cross was made between two strains, aecia arose within a few days in the pustules that received the exchanged nectar, the two strains were considered interfertile; if no aecia appeared, they were considered intersterile.

To serve as controls, haploid pustules of each strain were selfed, that is to say, composite nectar of a strain was applied to the upper surface of pustules of the same strain.

The data relative to the crosses made and the results obtained in this series of experiments are summarized in Table II. By way of explanation, it may be mentioned that, in this table, the arrow heads indicate the direction in

TABLE II

THE RESULTS OBTAINED FROM CROSSES MADE BETWEEN DIFFERENT STRAINS OF *P. Helianthi* ORIGINATING ON FOUR DIFFERENT SPECIES OF *Helianthus*

Crosses	Number of pustules with aecia
H. subtuberosus strain (23) ⇋ *H. petiolaris* strain (34)	0
H. subtuberosus strain (11) ⇋ *H. tuberosus* strain (10)	19
H. subtuberosus strain (29) ⇋ *H. annuus* strain (61)	0
H. tuberosus strain (22) ⇋ *H. petiolaris* strain (45)	0
H. tuberosus strain (62) ⇋ *H. annuus* strain (71)	0
H. petiolaris strain (16) ⇋ *H. annuus* strain (29)	42
H. annuus strain, selfed (44)	40
H. tuberosus strain, selfed (23)	21
H. subtuberosus strain, selfed (20)	19
H. petiolaris strain, selfed (26)	24

which the pycnial nectar was transferred, and the figures enclosed by brackets give the number of pustules that received the transferred nectar. For example, in the first reciprocal cross, composite nectar of the *H. subtuberosus* strain was transferred to 34 haploid pustules of the *H. petiolaris* strain, and composite nectar of the *H. petiolaris* strain to 23 pustules of the *H. subtuberosus* strain.

Although the number of pustules used in the crosses is relatively small, the results given in Table II indicate that there is at least a high degree of intersterility between the strain of *P. Helianthi* on *H. subtuberosus* and the one on *H. petiolaris* or the one on *H. annuus*, but a high degree of interfertility between the strain on *H. subtuberosus* and the one on *H. tuberosus*. The strain on *H. annuus* and the one on *H. petiolaris* are highly interfertile, but both are highly intersterile with the strain on *H. tuberosus*.

Additional tests were made with the *annuus* and *tuberosus* strains, each strain being cultured on its respective host. Of the two strains a total of 43 haploid pustules were crossed, but none produced aecia. Fifteen haploid pustules of the former strain and 12 of the latter were selfed. Aecia formed in 12 and 9 of the pustules, respectively.

B. By the Diploidisation Method

The validity of these results was tested by a second method of crossing the strains. It has been shown that, in the sunflower rust (3), a diploid mycelium will, when it interacts with a haploid mycelium, diploidise it and initiate the formation of aecia. This result was obtained with rust collected on the cultivated sunflower.

In the second series of experiments, haploid pustules of the four strains of rust were obtained on plants of Mammoth Russian sunflower. Each plant bore pustules of a single strain. The haploid pustules used in these tests were isolated on the leaves, and all were at least 21 days old. The method of making a cross was as follows. A leaf bearing a haploid pustule of one strain was inoculated near the periphery of the pustule with uredospores (diploid) of another strain. Uredia arose in close proximity to the haploid pustule and thus an opportunity was afforded for the interaction of the two types of mycelia. All possible combinations of haploid with diploid mycelia were made among the four strains of rust. If, after reciprocal pairing of haploid and diploid pustules of two strains, aecia were produced in the formerly haploid pustules, the two strains were considered interfertile, if no aecia appeared, intersterile (Plate I, C).

As a control for each cross, both parental strains were "selfed", that is to say, haploid and diploid pustules of the same strain were brought together, after the manner of making a cross described above.

The results of this series of experiments are summarized in Table III. With the exception of a slight deviation in the cross between the *annuus* and the

TABLE III

The results of pairing haploid with diploid mycelia in four strains of *P. Helianthi*

Haploid strain	Diploid strain	Number of haploid pustules	Number of pustules with aecia
H. annuus	*H. subtuberosus*	20	2
H. subtuberosus	*H. annuus*	26	1
H. annuus	*H. tuberosus*	23	0
H. tuberosus	*H. annuus*	74	0
H. petiolaris	*H. subtuberosus*	18	0
H. subtuberosus	*H. petiolaris*	7	0
H. tuberosus	*H. petiolaris*	6	0
H. petiolaris	*H. tuberosus*	10	0
H. annuus	*H. petiolaris*	34	31
H. subtuberosus	*H. tuberosus*	26	22
H. annuus	*H. annuus*	10	10
H. petiolaris	*H. petiolaris*	27	24
H. subtuberosus	*H. subtuberosus*	19	18
H. tuberosus	*H. tuberosus*	31	29

subtuberosus strains, the results of these experiments are in agreement with those of the first series. In the first series the *annuus* and the *subtuberosus* strains appeared to be perfectly intersterile, but, as shown in Table III, a slight degree of interfertility is indicated. Out of 46 pairings of haploid and diploid pustules, three of the haploid pustules produced aecia.

In these three pustules, the aecia were few in number and these few arose at the margins of the haploid pustules, just where they made contact with diploid pustules. The aecia were more or less rudimentary in appearance and viable aeciospores were recovered from only some of them. When two interfertile strains unite, aecia usually appear first near the point of contact between the haploid and the diploid pustule and then continue to appear progressively across the formerly haploid pustule until the whole undersurface is studded with aecia.

Aeciospores from these three pustules were used to inoculate Mammoth Russian plants. It was only on some plants that had been left in moist chambers for eight days that infections were secured. These infections were sparse. Apparently the aeciospores were but weakly viable. Uredospores produced by these infections were likewise subnormal with respect to viability.

A striking feature of the germination of these uredospores was the abnormal type of germ tube produced. Many of the germ tubes resembled the promycelia formed by teliospores of *P. Helianthi* germinated in hanging drops of water. Most of the germ-tube tips were swollen. Apical swelling in uredospore germ tubes is apparently not an uncommon phenomenon, for it was observed as early as 1854 by Tulasne (13) and later by other investigators. Ezekiel (6) found it common in physiologic races (forms) of *P. graminis Tritici*, but much more abundant in some races than in others. In the present case, however, it was observed only in the germ tubes of uredospores that were derived from the cross between the nearly intersterile *annuus* and *subtuberosus* strains of rust.

Discussion

The identity of the four strains of rust has not been determined on all the differentials used by Bailey (2). Only three of his differential species were available, namely, *H. annuus* (Mammoth Russian), *H. tuberosus* (probably his second strain of this species), and *H. Maximiliani*. There is no surety, however, that these representatives were genotypically identical, respectively, with the three that he worked with. The likelihood is that they were not; for, as he pointed out, sunflowers are practically self-sterile, so that each species must include a large number of genotypes, and, therefore, "the reactions indicated for the particular collection of the species worked with cannot be expected to hold for all samples of the same species". However, the strain collected on *H. annuus* produced the same types of infection on the three differentials of his mentioned above and is probably identical with his race (form) 1. The other three strains lacked agreement with both of his other races.

It has been shown in the present study that, on a basis of their interfertility, the four strains fall into two distinct groups, which, for convenience, may be called A and B. Group A consists of the two strains on *H. annuus* and *H. petiolaris*, and Group B of the two strains on *H. tuberosus* and *H. subtuberosus*. Arthur (1) concluded from his own work and that of others that, within the species *P. Helianthi*, "a number of physiologic forms exist, but none so distinctive that they could be called varieties". However, the similarity of the crossing behavior of the two strains in either Group A or Group B with that of races within a variety of *P. graminis*, and the similarity of the crossing behavior of the two groups, A and B, with that of the variety *Tritici* and the variety *Agrostidis* or *Avenae* of *P. graminis* suggest strongly that each group represents a variety.

To this concept there is the objection that the parallelism does not extend to the hosts. The varieties of *P. graminis* are more or less specialized to different genera, *e.g.*, the *Tritici* variety to wheat, the *Avenae* variety to oats, etc., whereas the two groups of *P. Helianthi* occur on species of the same genus. Moreover, one host, Mammoth Russian, is susceptible to the two strains of Group A, and to one strain (on *H. tuberosus*) of Group B. This statement, perhaps, requires some qualification. What actually happens is that, on Mammoth Russian, the strain collected on *H. tuberosus* produces a susceptible type of reaction on the upper surface of the leaves, but no uredia are formed on the lower surface. In susceptible species, uredia regularly form on the lower surface of the leaves. If the absence of uredia on the lower surface indicates resistance, Mammoth Russian can be considered resistant to both strains of Group B, in which case the parallelism is brought somewhat more closely into line.

As has been mentioned above, there seems to be considerable difference in the interfertility of different varieties of *P. graminis*. The crossing behavior of the two groups of *P. Helianthi* seems to resemble that of the *Tritici* variety and the *Agrostidis* or *Avenae* variety of *P. graminis*. Before drawing any definite conclusion as to whether these two groups of *P. Helianthi* represent different varieties, it would be well to await the results of crossing experiments with varieties of other rust fungi, such as varieties of *Puccinia coronata* Cda. or of *P. rubigo-vera* Carl. It is not, therefore, proposed here to raise the two groups of *P. Helianthi* to the rank of variety, and burden each with a trinomial designation, although further studies may show this to be necessary. For the time being, the data presented are given for what they are worth as a contribution to the point under discussion.

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INHERITANCE STUDIES OF SEVERAL QUALITATIVE AND QUANTITATIVE CHARACTERS IN SPRING WHEAT CROSSES BETWEEN VARIETIES RELATIVELY SUSCEPTIBLE AND RESISTANT TO DROUGHT[1]

By J. H. Torrie[2]

Abstract

Genetic studies were made in the F_2 and F_3 generations of the crosses, Selection I-28-60 × Milturum, Reward × Caesium, and Caesium × Marquis. It was found that the character glume color was controlled by either one or two factor pairs in the cross Selection I-28-60 × Milturum, and by two factor pairs in the crosses Reward × Caesium and Caesium × Marquis. The characters, awning, straw color, glume pubescence and spike regularity were each governed by one factor pair, while three factor pairs were operative in the inheritance of seed color.

Polymeric factors apparently control the inheritance of the quantitative characters straw strength, plant height, earliness and grain yield. A partial dominance of strong straw and earliness was found in the crosses Reward × Caesium and Caesium × Marquis. Tallness and low grain yield were partially dominant in the cross Reward × Caesium. Evidence for transgressive segregation of earliness was obtained in the cross between Caesium and Marquis.

The characters glume color, awning, straw color, glume pubescence and spike regularity were inherited independently. White straw color and earliness were definitely associated in the crosses Reward × Caesium and Caesium × Marquis. The characters straw color and plant height were loosely linked in the Caesium × Marquis cross. Grain yield was not significantly correlated with straw strength, plant height or earliness in the cross between Reward and Caesium. Small but significant relationships were found among the characters straw strength, plant height and earliness in the crosses Reward × Caesium and Caesium × Marquis. The relation between heading and maturity was studied only in the F_4 of Reward × Caesium, in which case a strong positive correlation was obtained.

Introduction

Drought is one of the major limiting factors in successful wheat production in most areas of western Canada. This has been particularly exemplified during the past five years. The development of a wheat possessing a measure of drought resistance would be a forward step in stabilizing wheat production. With this object in view, the University of Alberta, in 1929, in co-operation with the National Research Council of Canada, initiated a program of breeding drought resistant wheat (1, 2). The studies reported herein, on the genetics of several qualitative and quantitative characters, were undertaken as part of this program. Studies on the drought resistance (5) and quality (4) of these hybrids are being reported in separate papers.

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Materials and Experimental Methods

The material used for these studies consisted of the F_2 and F_3 generations of the crosses, Selection I-28-60 × Milturum, Reward × Caesium and Caesium × Marquis, and certain F_4 lines of Reward × Caesium. Reciprocal crosses were made only between the varieties Caesium and Marquis. Since no significant differences for any of the characters studied were obtained in the reciprocal crosses, the data were treated collectively. Table I gives a comparison of the parental varieties for the characters studied.

TABLE I

CHARACTERS OF PARENTAL VARIETIES STUDIED

Characters studied	Variety				
	Caesium 0.111	Milturum 0.321	Selection I-28-60	Marquis	Reward
C.A.N.*	1256	1415	—	1621	1509
Glume color	Brown	Brown	White	White	White
Glume pubescence	Glabrous	Glabrous	Glabrous	Glabrous	Pubescence
Awning	Awned	Awnletted	Awnletted	Awnletted	Awnletted
Spike regularity	Irregular	Regular	Regular	Regular	Irregular
Straw color	Purple	Purple	White	White	White
Seed color	Red	Red	Red	Red	Red
Strength of straw	Weak	Weak	Strong	Strong	Strong
Height of plant	Tall	Tall	Short	Medium	Short
Maturity	Late	Late	Medium	Medium	Early

**C.A.N. Canadian Accession Number.*

Caesium 0.111 and Milturum 0.321 are red spring wheats obtained, in 1928, from Dr. Talanoff of the West Siberian Plant Breeding Station at Omsk, U.S.S.R. Talanoff (29) states that under conditions of extreme drought these two wheats proved to be the most productive and drought resistant of all the varieties tested. Selection I-28-60, Marquillo × (Marquis-Kanred) is a hard red spring wheat, selected at the University of Alberta from a strain introduced in 1928 from the University of Minnesota. Marquis and Reward are hard red spring wheats which originated at the Central Experimental Farm, Ottawa.

The F_2 lines of all crosses and the F_3 lines of Selection I-28-60 × Milturum were planted in five-foot rows, approximately 25 seeds being planted in each row. The F_3 and F_4 lines of Reward × Caesium and the F_3 lines of Caesium × Marquis were planted in single ten-foot rows, approximately 50 seeds being planted in each row. Parental varieties were sown at intervals of approximately 30 rows.

The characters studied were placed in two groups; qualitative and quantitative. Those treated qualitatively were classified into definite categories. The qualitative characters are glume color, awning, straw color, seed color, glume pubescence and spike regularity. The quantitative characters which were classified by arbitrary numerical values are straw strength, plant height, earliness and grain yield.

The methods of handling and classifying the material, for any individual character, are presented later with the discussion of that character. Data relative to F_2 populations derived from different F_1 plants were computed separately. These were combined after it was ascertained that there were no significant differences between the several F_2 populations. The methods described by Fisher (14) were used in the analysis of the data.

Inheritance of Qualitative Characters

Table II shows the breeding behavior of glume color in the cross Selection I-28-60 × Milturum. The breeding behavior of the Reward × Caesium cross for the characters, glume color, awning, straw color, seed color and glume pubescence is given in Table III. Table IV presents the breeding behavior of Caesium × Marquis for the characters, glume color, awning, straw color, seed color and spike regularity. In these three tables the data obtained are given for each character for the F_2 segregation based on their F_3 breeding behavior. These are followed by the data for the segregation of the plants in the F_3 from heterzygous F_2 plants.

TABLE II

THE BREEDING BEHAVIOR OF SELECTION I-28-60 × MILTURUM CROSS FOR GLUME COLOR AND TESTS OF GOODNESS OF FIT

Number of F_3 lines or plants	Expected ratio	Observed	Calculated	χ^2	P lies between
170 lines	1 : 2 : 1	40 : 88 : 42	42.5 : 85.0 : 42.5	0.27	.90 and .80
1711 plants	3 : 1	1269 : 442	1283.3 : 427.8	0.64	.50 and .30
167 lines	7 : 4 : 4 : 1	86 : 26 : 43 : 12	73.1 : 41.8 : 41.8 : 10.4	8.50	.05 and .02
710 plants	15 : 1	660 : 50	665.6 : 44.4	0.76	.50 and .30
1261 plants	3 : 1	936 : 325	947.8 : 315.2	0.40	.70 and .50

Glume Color

In several crosses of *T. vulgare* wheats, Biffen (7) found that the difference between brown and white glume color is monogenic, the brown glume color being dominant. In crosses made between some white-chaffed and brown-chaffed Swedish "land wheats", Nilsson-Ehle (24) obtained in the F_2 a ratio of 3 brown : 1 white; or in other cases 15 brown : 1 white.

In the present investigation the inheritance of glume color was studied in all three crosses. Variation was noticed in the pigmentation of the brown glume. Since this was believed to be influenced considerably by environmental factors, the plants were classified into two general classes, brown and white. No difficulty was experienced in distinguishing the plants falling into these two categories.

Eight F_2 populations from the Selection I-28-60 × Milturum cross were monogenic, while seven were dihybrids for the expression of glume color. Table II gives the observed and calculated ratios. Satisfactory fits to the theoretical ratios were obtained in all cases save for the F_2 populations

segregating into the dihybrid ratio 7 : 4 : 4 : 1. A P value lying between .05 and .02 was obtained in this instance. The poor fit is largely due to an excess of homozygous brown lines and a corresponding deficiency of lines segregating into the 15 : 1 ratio. This is explainable on the assumption that the number of plants in each F_3 line (20–25) was too few to obtain segregation in all the F_3 lines heterozygous for two-factor pairs.

In the crosses Reward × Caesium and Caesium × Marquis, two independent factor pairs were found to be operative in the inheritance of glume color. The experimental data are given in Tables III and IV.

Awning

The literature on the inheritance of awning in wheat has been recently reviewed by Kilduff (23). In crosses between awnletted and fully awned wheats, a single-factor-pair difference has been found by practically all investigators. In a cross Sonora × Reliance, Clark and Quisenberry (13) obtained in the F_3 too few awnletted lines and an excess of segregating lines. The segregating lines had a great excess of awned plants which, the authors suggest, may be the result of the presence of a dominant factor-pair for the awned condition. In a Kota × Garnet cross, Kilduff (23) found a very poor fit for the one-factor-pair hypothesis. He obtained an excess of true breeding fully awned lines, which he believes is due to more than one factor pair operating in this cross.

Awning was studied in the crosses Reward × Caesium and Caesium × Marquis. The awnletted class had a considerable range in its degree of expression. The difference, however, between the awnletted and awned plants was clear cut. The difference between the fully awned condition of Caesium and the awnletted condition of Reward and Marquis was found to be monogenic. This is shown by the data in Tables III and IV. A poor fit to the theoretical ratio was obtained in the F_3 segregating lines of Caesium × Marquis. The poor agreement was caused by excess of awned plants. No satisfactory genetic explanation can be offered to account for this discrepancy.

Straw Color

An examination of the available literature of inheritance in wheat revealed no instance reporting the inheritance of straw color. This character was studied in the crosses of Caesium with Reward and Marquis. On account of a marked bleaching of the purple color, some difficulty was experienced in the F_2 classification of the culm color for certain plants. In this respect very little difficulty occurred in the F_3. The upper leaf sheath was removed from the plants of which the culm color was doubtful. This resulted in the uncovering of an unbleached portion of the culm. No great difficulty was experienced in distinguishing between bleached purple and white culms. In both crosses, as shown by the data in Tables III and IV, the difference between purple and white straw color is dependent upon one factor pair. Purple straw color was partially dominant.

TABLE III

THE BREEDING BEHAVIOR OF REWARD × CAESIUM CROSS FOR SEVERAL QUALITATIVE CHARACTERS, AND TESTS OF GOODNESS OF FIT

Character	Number of F_3 lines or plants	Expected ratio	Observed	Calculated	χ^2	P lies between
Glume color	132 lines	7 : 4 : 4 : 1	53 : 28 : 38 : 13	57.8 : 33 : 33 : 8.2	4.68	.20 and .10
Glume color	1059 plants	15 : 1	988 : 71	992.8 : 66.2	0.37	.70 and .50
Glume color	1612 plants	3 : 1	1213 : 399	1209 : 403	0.53	.90 and .80
Awning	132 lines	1 : 2 : 1	29 : 71 : 32	33 : 66 : 33	0.89	.70 and .50
Awning	2886 plants	3 : 1	2129 : 757	2164.5 : 721.5	2.31	.20 and .10
Straw color	132 lines	1 : 2 : 1	28 : 77 : 27	33 : 66 : 33	3.68	.20 and .10
Straw color	3175 plants	3 : 1	2401 : 774	2381.2 : 793.8	0.65	.50 and .30
Seed color	132 lines	37 : 8 : 12 : 6 : 1	89 : 8 : 21 : 11 : 3	76.3 : 16.5 : 24.7 : 12.4 : 2.1	7.55	.20 and .10
Seed color	341 plants	63 : 1	333 : 8	335.7 : 5.3	1.34	.30 and .20
Seed color	835 plants	15 : 1	780 : 55	782.8 : 52.2	0.16	.70 and .50
Seed color	452 plants	3 : 1	341 : 11	339 : 113	0.04	.90 and .80
Glume pubescence	132 lines	1 : 2 : 1	31 : 64 : 37	33 : 66 : 33	0.94	.70 and .50
Glume pubescence	2557 plants	3 : 1	1808 : 749	1917.7 : 639.3	25.15	.01 and .00

TABLE IV

THE BREEDING BEHAVIOR OF CAESIUM × MARQUIS CROSS FOR SEVERAL QUALITATIVE CHARACTERS, AND TESTS OF GOODNESS OF FIT

Character	Number of F_3 lines or plants	Expected ratio	Observed	Calculated	χ^2	P lies between
Glume color	275 lines	7 : 4 : 4 : 1	116 : 68 : 66 : 25	120.3 : 68.8 : 68.8 : 17.2	3.90	.30 and .20
Glume color	2671 plants	15 : 1	2471 : 200	2504.1 : 176.9	1.06	.30 and .20
Glume color	2730 plants	3 : 1	2075 : 655	2047.5 : 682.5	1.48	.30 and .20
Awning	296 lines	1 : 2 : 1	79 : 131 : 86	74 : 148 : 74	3.24	.20 and .10
Awning	5328 plants	3 : 1	3782 : 1546	3996 : 1332	41.08	.01 and .00
Straw color	296 lines	1 : 2 : 1	83 : 137 : 76	74 : 148 : 74	1.96	.50 and .30
Straw color	5714 plants	3 : 1	4317 : 1397	4285.5 : 1428.5	0.92	.50 and .30
Seed color	274 lines	37 : 8 : 12 : 6 : 1	185 : 23 : 37 : 22 : 7	158.4 : 34.2 : 51.4 : 25.7 : 4.3	14.47	.01 and .00
Seed color	939 plants	63 : 1	916 : 23	924.3 : 14.7	4.81	.05 and .02
Seed color	1453 plants	15 : 1	1355 : 98	1352.2 : 90.8	0.57	.50 and .30
Seed color	887 plants	3 : 1	686 : 201	665.3 : 221.7	2.59	.20 and .10
Spike regularity	296 lines	1 : 2 : 1	57 : 172 : 67	74 : 148 : 74	8.46	.02 and .01
Spike regularity	6895 plants	3 : 1	5027 : 1868	5172 : 1724	16.00	.01 and .00

Seed Color

Kilduff (23) has recently reviewed the literature on the inheritance of seed color in wheat. Previous investigators have shown that one, two or three factor pairs govern the inheritance of this character.

The inheritance of seed color was studied in crosses of the variety Caesium with Reward and Marquis. Three factor pairs were found to govern the expression of seed color in these crosses. The observed and calculated numbers are given in Tables III and IV. In the F_3 of Caesium × Marquis a P value less than .01 was obtained. The population of an F_3 line (45–50) plants was too small to obtain segregation in all the lines heterozygous for the tri-hybrid ratio.

In the cross Caesium × Marquis, two F_2 populations with more than 1000 F_3 plants, gave no white seeded segregates. In this case one of the factor pairs for red seed color of Marquis and that of Caesium are allelomorphic.

Glume Pubescence

Biffen (7) found that the difference between glabrous and pubescent glumes was monogenic in a pubescent *T. turgidum* × glabrous *T. vulgare* and also in an intra *T. vulgare* cross. In several crosses of pubescent *T. durum* × glabrous *T. vulgare* and pubescent *T. vulgare* and glabrous *T. vulgare*, Howard and Howard (22), obtained an intermediate condition in the F_1 and a ratio of 15 pubescent : 1 glabrous in the F_2.

A one factor pair difference was found between pubescent and glabrous glumes in the cross Reward × Caesium. The data are given in Table III. A P value less than .01 was obtained in the segregating F_3 lines. This poor agreement is largely on account of the excess of glabrous plants, and a corresponding deficiency of pubescent ones. A considerable range was noticed in the degree of glume pubescence. This was probably due to the intermediate heterozygous condition, which made it difficult to distinguish between slightly pubescent and glabrous plants. It is also possible that the pubescence of a number of slightly pubescent plants may have been brushed off in handling, thus making it difficult to distinguish them from glabrous plants. For these reasons it is believed that the excess of glabrous plants resulted from the classification of certain pubescent plants as glabrous.

Spike Regularity

An examination of the available literature of the inheritance in wheat revealed no case reporting the inheritance of spike regularity.

For the character of spike regularity, the plants were classified into two categories, regular and irregular. The regular spike refers to the typical spike arrangement found in most common wheat varieties, in which the spikelets are arranged more or less parallel to the rachis from a lateral view, thus giving the spike a uniform appearance or regular outline. The irregular spike condition refers to the arrangement found in Reward and Caesium,

in which case most of the spikelets are arranged at a distinct angle to the rachis, thus giving an unsymmetrical or irregular appearance to the spike. These are shown in Fig. 1.

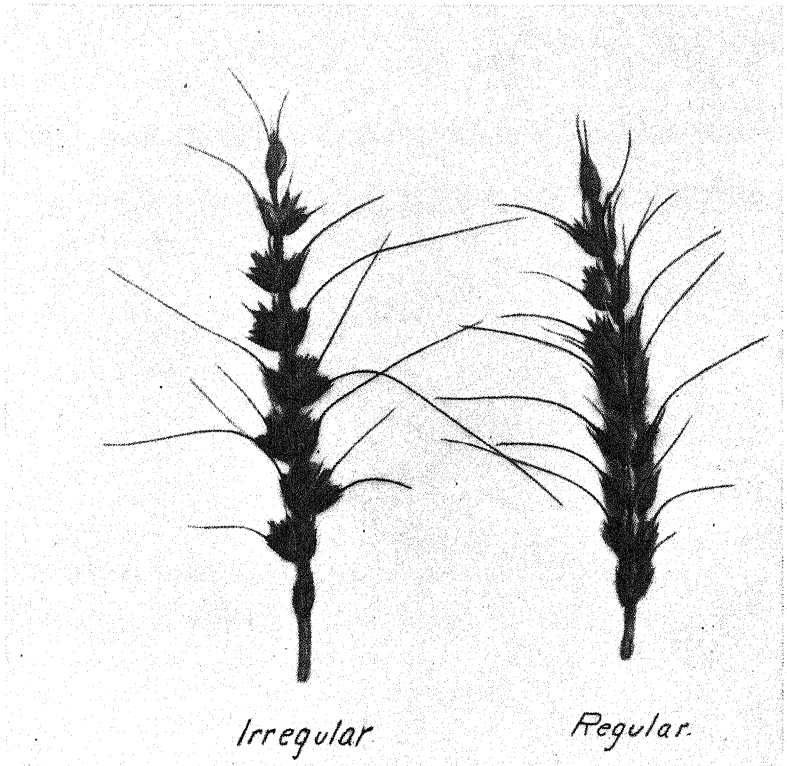

FIG. 1. *Irregular and regular spike.*

Table IV shows the breeding behavior of this character in the cross Caesium × Marquis. Although poor fits were obtained for the one factor pair hypothesis, it is quite evident that no other simple genetic scheme would fit the data. Some difficulty was experienced in the classification of certain plants, owing to an intermediate expression of this character. The intermediate condition resulted from a partial dominance of the irregular spike.

Inheritance of Quantitative Characters

Strength of Straw

The ability of wheat to resist lodging is a very important agronomic character. Strength of straw is influenced considerably by environmental factors which render a genetical study very difficult. Kilduff (23) has recently reviewed the literature on the inheritance of this character. Harrington (20) and Kilduff (23) both suggest that several factor pairs govern the inheritance of straw strength.

Strength of straw was studied in the F_3 of Caesium crossed with Reward and Marquis and in certain F_4 lines of Reward × Caesium. A satisfactory differentiation for straw strength was secured for all the material studied. An estimation of straw strength was obtained for each line by the lodging index. This index was calculated by the formula:

$$\frac{\%\ \textit{plants lodged} \times \textit{average angle off the vertical}}{90}$$

Earliness of Heading

Biffen (7) and Florell (15), both report that one factor pair governs the inheritance of earliness in wheat. Florell (15) suggests, however, the possible presence of a number of minor modifying factor pairs. Nilsson-Ehle (25), Thompson (30), Harrington (20), Stephens (28) and Florell (16), all suggest that two or more factor pairs are present. Transgressive segregation for earliness was found by Thompson (30), Bryan and Pressley (9), Harrington (20) and Clark and Hooker (12). Fruwirth, cited by Florell (15), Florell (15), Clark (10) and Stephens (28) report the dominance or partial dominance of earliness. On the other hand, Freeman (17) and Bryan and Pressley (9) found a dominance or partial dominance of lateness.

Environmental as well as genetical factors play an important role in determining the growth period of wheat varieties. Date of heading is less influenced by environmental factors than is date of maturity in any given season. For this reason, it is a better indication of earliness, in genetical studies, than is date of maturity. The emergence of the first spike from the sheath was used as the index of heading. Earliness was studied in the F_3 of Caesium crossed with Reward and Marquis and in selected F_4 lines of Reward × Caesium. During the heading period, for all the material studied, the weather was ideal, no heavy rains occurring to prevent the taking of daily notes. In the F_3 of Reward × Caesium, in half of the population the plants were tagged daily as they headed, while in the remainder of the population the total number of plants headed for each line was counted each day. In the F_3 of the cross Caesium × Marquis and the F_4 of Reward × Caesium the dates when the first plant headed, approximately 50% of the plants, and the last plant headed, were recorded for each line. The two methods were found to check very closely when applied to the F_3 lines of the cross between Reward and Caesium.

The distribution, for days from seedling emergence to heading, of the means for the F_3 and parental lines of Caesium crossed with Reward and Marquis are given in Table VII. The difference between Reward and Caesium of 9.3 ± 0.25 days and between Caesium and Marquis of 3.2 ± 0.22 days are both very significant. The distribution of the means of the F_3 lines in Reward × Caesium approaches a normal curve with a slight tendency to mass towards the mean of the early parent. Further studies in the F_4 of this cross showed that apparent homozygous lines occurred at several points intermediate between the means of the two parental varieties.

TABLE V

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR LODGING INDEX OF REWARD × CAESIUM AND CAESIUM × MARQUIS F_3 AND PARENTAL LINES

Parent or cross	Lodging index in 5 per cent classes												Total number	Mean	Standard deviation
	0	3	8	13	18	23	28	33	38	43	48	53			
Reward	3	9	–	–	–	–	–	–	–	–	–	–	12	2.3±0.37	1.3±0.27
Caesium	–	–	–	–	–	–	–	2	6	4	–	–	12	38.9±0.65	2.3±0.47
Reward × Caesium	11	42	24	17	14	7	6	2	5	4	–	–	132	11.6±1.00	11.4±0.70
Marquis	7	20	2	1	–	–	–	–	–	–	–	–	30	3.0±0.49	2.7±0.34
Caesium	–	–	–	–	–	–	–	5	5	8	5	7	30	43.7±1.28	7.0±0.89
Caesium × Marquis	30	154	240	207	224	169	100	116	60	27	41	26	1394	18.9±0.37	13.7±0.25

TABLE VI

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR HEIGHT OF PLANT OF REWARD × CAESIUM AND CAESIUM × MARQUIS F_3 AND PARENTAL LINES

Parent or cross	Plant height in 3 cm. classes												Total number	Mean	Standard deviation
	104	107	110	113	116	119	122	125	128	131	134	137			
Reward	–	–	3	8	1	–	–	–	–	–	–	–	12	112.5±0.49	1.7±0.24
Caesium	–	–	–	–	–	–	–	–	1	9	2	–	12	131.2±0.43	1.5±0.31
Reward × Caesium	1	1	–	1	4	12	32	26	26	16	11	2	132	125.3±0.48	5.4±0.34
Marquis	–	–	1	3	6	–	–	–	–	–	–	–	10	114.5±0.64	2.0±0.45
Caesium	–	–	–	–	–	–	–	1	–	4	3	2	10	132.5±1.07	3.4±0.76
Caesium × Marquis	–	1	2	8	21	44	67	70	56	18	8	2	297	123.6±0.28	5.0±0.21

TABLE VII

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR DAYS FROM SEEDLING EMERGENCE TO HEADING OF REWARD × CAESIUM AND MARQUIS × CAESIUM F_3 AND PARENTAL LINES

Parent or cross	Days from emergence to heading in one-day classes															Total number	Mean	Standard deviation
	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65			
Reward	3	6	3	–	–	–	–	–	–	–	–	–	–	–	–	12	52.0±0.21	0.71±0.15
Caesium	–	–	–	–	–	–	–	–	–	–	9	3	–	–	–	12	61.3±0.14	0.44±0.09
Reward × Caesium	–	4	7	19	23	29	18	14	10	4	3	1	–	–	–	132	56.1±0.18	2.11±0.13
Marquis	–	–	–	–	1	4	13	12	–	–	–	–	–	–	–	30	57.2±0.13	0.79±0.10
Caesium	–	–	–	–	–	–	–	1	5	9	12	3	–	–	–	30	60.4±0.18	0.94±0.12
Caesium × Marquis	–	5	35	97	186	280	278	224	150	76	37	24	16	3	1	1412	57.0±0.06	2.11±0.04

The F_3 distribution of Caesium × Marquis also approaches that of a normal curve, the mean being approximately equal to that of Marquis. Transgressive segregation for time of heading occurred beyond the extremes of both parents, but was, however, considerably more pronounced in the direction of Marquis. These data show a partial dominance of earliness and that genes for this character were contributed by both parental varieties. Polymeric factors are indicated as governing the inheritance of earliness in both crosses.

Five F_3 plants from each of 34 F_4 lines of the cross Reward × Caesium, were studied in the F_4 for earliness. The F_3 plants selected, from each F_3 line used, were representative of the earliest, intermediate and latest individuals in the line. The F_3 lines used were chosen at random. A correlation coefficient of $+.652$ was found between the individual F_3 plants and the corresponding F_4 lines, while an r of $+.781$ was obtained for the correlation between the average of the five F_3 plants grown in the F_4 and the mean of the F_3 lines from which the plants were selected. The P values of both correlations exceeded the 1% point.

Grain Yield

Aamodt, Torrie and Wilson (6) have recently reviewed the literature on the inheritance of grain yield in wheat. The results of previous investigators show that grain yield is a definite heritable character, but that its expression is influenced to a large extent by environmental conditions.

The plant yield of grain in grams was studied in the F_3 and F_4 in the cross Reward × Caesium. The plants of each line were harvested separately. The heads were wrapped in cheesecloth to prevent loss in handling. The border plants, at both ends of each row, were noted but were not used in the yield studies. Each plant was threshed separately and the yield recorded in grams. The average grain yield per plant and standard deviation were calculated for each line.

The distributions of the mean grain yield per plant for the F_3 and parental lines of Reward × Caesium are shown in Table VIII. The standard deviation and standard errors were not calculated for the parental varieties, on account of the small number of lines used. The F_3 distribution is in the form of a

TABLE VIII

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR GRAIN YIELD PER PLANT OF REWARD × CAESIUM F_3 AND PARENTAL LINES

Parent or cross	Grain yield per plant in 0.5 gram classes											Total number	Mean	Standard deviation
	5.8	6.3	6.8	7.3	7.8	8.3	8.8	9.3	9.8	10.3	10.8			
Reward	–	2	2	1	–	–	–	–	–	–	–	5	6.7	—
Caesium	–	–	–	–	–	–	–	–	1	1	3	5	10.5	–
Reward × Caesium	1	1	6	13	11	11	6	5	2	1	–	57	8.0 ± 0.12	0.93 ± 0.09

normal curve. The mean is inclined towards that of Reward, the low-yielding parent. The data suggest that polymeric factors are concerned in the difference between the yielding ability of Reward and Caesium.

Five F_3 plants from each of 15 F_3 lines, selected at random, were studied in the F_4. The F_3 plants, selected from each F_3 line, were representative of the lowest, intermediate and highest yielding individuals in the line. A value of $+.279$ was secured for the correlation coefficient between the grain yield of the individual F_3 plant and that of the corresponding F_4 line. A much more significant value of $+.648$ was obtained between the average of the plants grown in the F_4 and the mean of the F_3 line from which they were selected. The P value of the first correlation exceeded the 5% point, while that of the second exceeded the 1% point. The higher value of the second correlation can be explained largely by the fact that the average grain yield of all the plants within a line gives a much better indication of yielding ability than that of any individual plant. This is largely on account of the great variability in grain yield that occurs among the individual plants in a line.

Correlated Inheritance

The relationships of the qualitative characters with each other, and with those of the quantitative characters, were measured by the χ^2 test of independence or association. The association between any two characters was obtained by calculating χ^2 and determining its value from Fisher's tables (14). Simple and partial correlation coefficients were used to study the relationships among the quantitative characters. Throughout these analyses a P value of .05 was taken as the level of significance.

Table IX gives the χ^2 tests of independence or association among the qualitative characters studied in the F_3 of the crosses Reward × Caesium and Caesium × Marquis. The data in this table show that the characters are inherited independently.

TABLE IX

χ^2 TESTS OF INDEPENDENCE OR ASSOCIATION AMONG THE QUALITATIVE CHARACTERS STUDIED IN THE F_3 OF THE CROSSES REWARD × CAESIUM AND CAESIUM × MARQUIS

Characters compared*	Reward × Caesium		Caesium × Marquis	
	χ^2	P lies between	χ^2	P lies between
Awning and glume color	10.46	.20 and .10	7.91	.20 and .10
Awning and glume pubescence	7.28	.20 and .10	—	—
Awning and straw color	5.53	.30 and .20	4.94	.30 and .20
Awning and spike regularity	—	—	2.73	.70 and .50
Glume pubescence and straw color	4.63	.50 and .30	—	—
Glume pubescence and glume color	3.63	.80 and .70	—	—
Straw color and glume color	4.70	.50 and .30	5.64	.50 and .30
Straw color and spike regularity	—	—	0.99	.95 and .90
Glume color and spike regularity	—	—	8.33	.30 and .20

**The degrees of freedom used for the different comparisons were not necessarily the same.*

Aamodt and Torrie (3) have recently reviewed the literature on the relation between awning and grain yield in wheat. Certain investigators found an association between these two characters, while others found no association, depending both upon the material used and the environmental conditions under which it was grown. In the crosses Bobs × Propo, and Hard Federation × Propo, Clark, Florell and Hooker (11) found a positive relation between lodging and awn length. Goulden and Neatby (19) obtained no relation between awnedness and either straw strength or plant height, from a study of H-44-24 × Marquis lines in rod row trials. However, they secured a P value of .029 between awning and earliness. In a cross between the varieties Forward and Albit, Shen (26) found no indication of a linkage between awning with either plant height or date of heading.

Table X gives the χ^2 tests of independence or association for the qualitative characters compared with the quantitative characters in the crosses Reward × Caesium and Caesium × Marquis. Straw color and earliness of heading showed definite indications of linkage in both crosses. In the F_3 and F_4 of the Reward × Caesium cross, the average number of days from seedling emergence to heading of the white strawed lines was respectively 1.6 ± 0.53 and 2.0 ± 0.50 days less than that for the purple strawed lines. The white strawed lines of Caesium × Marquis were 2.4 ± 0.29 days earlier than the purple strawed lines. In maturity the white strawed lines were 1.6 ± 0.35 days earlier than the purple strawed lines in the F_4 of Reward × Caesium. The average length of the white strawed lines was 3.6 ± 0.80 cm. shorter than the purple strawed lines in the F_3 of the cross Caesium × Marquis. No such relationship was found in the cross Reward × Caesium. This indicates that the genetical constitution of Reward and Marquis for plant height differs.

The awned F_4 lines of Reward × Caesium showed $4.3 \pm 1.7\%$ more lodging and were 1.0 ± 0.44 days earlier in heading than the awnletted lines, the P values in both of these cases being between .05 and .02. In the F_3 no significant association was found between these characters. For these reasons, the association found in the F_4 is not considered to be significant. The F_3 awned lines of Caesium × Marquis were 2.0 ± 0.80 cm. shorter than the awnletted lines. This difference is believed to be due to chance, for Caesium, the awned parent, is taller than Marquis, the awnletted parent. Additional factors for tallness could not have been contributed by Marquis as no transgressive segregation for plant height occurred in the F_3. No significant associations were found among the other characters studied.

Only a brief review of the results of other workers, concerning the relationships among the characters given in Table XI, will be made. Clark (10) Goulden and Elders (18), and Bridgford and Hayes (8) report significant positive correlations between grain yield and earliness. Significant negative correlations were obtained by Smith and Clark (27), Goulden and Neatby (19) and Waldron (31). No significant associations were found between these two characters by Clark and Hooker (12) and Hayes, Aamodt and Stevenson

TABLE X

χ^2 TESTS OF INDEPENDENCE OR ASSOCIATION FOR THE COMPARISON OF THE QUALITATIVE WITH THE QUANTITATIVE CHARACTERS OF THE F_3 AND F_4 OF REWARD × CAESIUM AND THE F_3 OF CAESIUM × MARQUIS

Qualitative characters	Quantitative characters									
	Lodging index		Plant height		Grain yield		Days from emergence			
							To heading		To maturity	
	χ^2	P lies between	χ^2	P lies between	χ^2	P lies between	χ^2	P lies between	χ^2	P lies between
					F_3 Reward × Caesium					
Glume color	14.63	.30–.20	12.73	.20–.10	3.69	.95–.90	13.93	.50–.30	—	—
Glume pubescence	1.08	.98–.95	8.06	.20–.10	6.23	.30–.20	8.57	.50–.30	—	—
Straw color	5.40	.80–.70	1.57	.80–.70	5.68	.30–.20	17.27	.01–.00	—	—
Awning	8.70	.50–.30	10.40	.10–.05	7.65	.30–.20	6.52	.70–.50	—	—
					F_4 Reward × Caesium					
Glume color	2.50	.50–.30	5.40	.20–.10	2.60	.30–.20	0.50	.95–.90	0.73	.90–.80
Glume pubescence	2.42	.70–.50	2.53	.50–.30	2.27	.50–.30	4.94	.10–.05	1.69	.70–.50
Straw color	6.85	.20–.10	7.11	.10–.05	1.81	.50–.30	21.41	.01–.00	16.11	.01–.00
Awning	9.09	.05–.02	3.80	.30–.20	1.61	.50–.30	8.85	.05–.02	6.65	.10–.05
					F_3 Caesium × Marquis					
Glume color	15.68	.20–.10	4.46	.90–.80	—	—	7.18	.70–.50	—	—
Spike regularity	12.70	.20–.10	9.98	.20–.10	—	—	11.81	.20–.10	—	—
Straw color	5.01	.80–.70	23.65	.01–.00	—	—	63.78	.01–.00	—	—
Awning	6.68	.70–.50	14.43	.05–.02	—	—	7.62	.30–.20	—	—

The degrees of freedom used for the different comparisons were not necessarily the same.

(21). Between the characters of grain yield and plant height Clark (10), Clark and Hooker (12), Hayes, Aamodt and Stevenson (21), Goulden and Neatby (19) and Bridgford and Hayes (8) obtained significant positive correlations, while Waldron (31) found no association. Goulden and Elders (18) found weak straw to be associated with high grain yield. Correlation studies between earliness and plant height have shown different results. Clark (10) Hayes, Aamodt and Stevenson (21) and Goulden and Neatby (19), obtained significant positive correlations; Bridgford and Hayes (8) significant negative correlations; while Clark (10) and Clark and Hooker (12) report the lack of significant correlations between these two characters. Goulden and Elders (18) and Goulden and Neatby (19) found no significant association between earliness and lodging. A significant negative correlation was obtained between plant height and straw strength by Goulden and Neatby (19). In many of the cases reported above, the apparent contradictory results found can be explained by the fact that the investigators worked with different material grown under widely differing conditions.

TABLE XI

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS AMONG THE QUANTITATIVE CHARACTERS OF THE F_3 AND F_4 OF REWARD × CAESIUM AND OF THE F_3 OF CAESIUM × MARQUIS

Simple			Partial		
Variables correlated†	Number	r	Variables correlated	Number	r
		F_3 Reward × Caesium			
YH	57	+.101			
YHe	57	+.162			
YL	57	+.054			
HHe	132	+.284**	HHe : L	132	+.290**
HL	132	+.191*	HL : He	132	+.161*
HeL	132	+.252**	HeL : H	132	+.253**
		F_4 Reward × Caesium			
YH	78	−.239*			
YHe	78	+.121			
YL	78	+.190			
YM	78	+.042			
HHe	145	+.560**	HHe : LM	145	+.124
HL	145	+.142	HL : HeM	145	−.219*
HM	145	+.782**	HM : HeL	145	+.677**
HeL	145	+.402**	HeL : HM	145	+.277**
HeM	145	+.676**	HeM : HL	145	+.376**
LM	145	+.327**	LM : HHe	145	+.207*
		F_3 Caesium × Marquis			
HHe	297	+.379**	HHe : L	297	+.416**
HL	297	+.216**	HL : He	297	+.171**
HeL	297	+.291**	HeL : H	297	+.314**

†*Y = grain yield in grams;* H = *days from emergence to heading;* He = *plant height;* L = *lodging index;* M = *days from emergence to maturity.*

*P *value exceeds 5% point.*

**P *value exceeds 1% point.*

Table XI gives the simple and partial correlations, among the quantitative characters studied, in the F_3 and F_4 of Reward × Caesium and in the F_3 of Caesium × Marquis. In the F_3 and F_4 of the cross Reward × Caesium, grain yield was not significantly correlated with any of the other characters, save with the one exception of earliness of heading in the F_4. In this case a significant negative correlation of $-.239$ was obtained. For these reasons the character grain yield was not included in the calculation of the partial correlations. Significant positive correlations were obtained in almost every other case. Between earliness of heading and lodging index significant positive correlations were obtained in the F_3 of both crosses. A significant negative correlation was found between these two characters in the F_4 of the cross Reward × Caesium. The selection of the F_3 plants, for F_4 planting, was at random. The differences in relationship found must be due either to a different effect of the environmental conditions of the two seasons, or to the holding of maturity constant in the calculation of the partial correlation in the F_4. The correlations between heading and height were significant in the F_3 of both crosses. In the F_4 of Reward × Caesium the second order partial correlation between these characters was positive but not significant. These correlations, although for the most part significant, show that the characters, with the exception of heading and maturity, are not closely dependent upon one another. The characters heading and maturity, which were studied only in the F_4 of Reward × Caesium, are shown to be closely related to one another.

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STATISTICAL SIGNIFICANCE OF WHEAT PROTEIN PERCENTAGE DIFFERENCES IN VARIETAL TRIALS[1]

BY A. G. O. WHITESIDE[2]

Abstract

The results of protein determinations on 28 varieties of spring wheat grown in quadruplicate rod-row plots at each of three Dominion Experimental Stations were analyzed statistically. The error due to plot variability greatly exceeded the laboratory error. No real differences were found between the calculated percentages for composite samples made up from the four plots of each station and the percentages obtained by averaging the results from the individual plots.

Some of the varieties showed definite tendencies towards high protein content. The major environmental effects of station and of replication gave negative correlations between yield and protein content but when these major factors together with the influence of variety were removed, yield and protein content were not correlated.

Introduction

In the relative classification of wheat varieties on the basis of wheat protein percentage, a knowledge of the accuracy of the test is important. Numerous investigators have pointed out that the quantity of protein found in wheat is greatly influenced by environment, and those familiar with appraising the relative quality of wheat varieties recognize that good field plot technique is as important for quality comparisons as it is for yield comparisons.

Newton and Malloch (1) demonstrated that wide variations may occur in the protein content of wheat grown in replicate plots and that single plots were unreliable for making varietal comparisons. In a series of 12 varieties grown at Raymond, Alberta, in 1/200 acre plots with four plots allotted to each variety, an average spread of 3.2% was found between the high and the low values for the plots of each variety. In another series of plots grown at Edmonton the average spread for all varieties was 1.1%. They conclude that "whatever method of replication or sampling is used, however, it is unsafe to attach importance to small differences unless these can be proven statistically to be significant."

In recent years plant breeders in Canada have more or less adopted the rod row method of variety testing first used in the United States. The plots are small in size and where close comparisons are desired the varieties in a group are few in number in order not to spread the experiment over too great an area of land. Varietal competition and border effect are taken care of by

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including only the inner rows of a plot and excluding the end plants of each row at harvest. Usually four replications are used for each variety and the manner of plot arrangement is such that Fisher's (2) analysis of variance may be applied to the data.

In 1935 a series of 28 varieties of spring wheat was grown in replicated rod row plots at each of three Dominion Experimental Stations. These are located at Swift Current and Scott, Saskatchewan, and at Lacombe, Alberta. Each plot consisted of 5 rows, 7 inches apart and 18½ ft. long. The plots of the 28 varieties were randomized within a replication and 4 replications were grown at each station. The grain harvested from each plot was taken from the 3 inner rows which measured 16½ ft. in length. The protein determinations were made by the Chemistry Division.

Laboratory Error

Fifty-gram samples were finely ground in a Hobart grinder; the protein percentages were determined in duplicate on each sample and the results calculated to 13.5% moisture basis. There were 112 samples from each station making 336 samples in all.

The statistical analysis of the data (Table I) indicated that protein percentages may be determined in the laboratory within narrow limits of variation. A difference of approximately 0.3 in percentage protein is necessary in order to be statistically significant in this series. It might be pointed out that the difference in the laboratory error for the three series is not significant, thus justifying the calculation of a single standard error for the entire series of determinations.

TABLE I

LABORATORY ERROR FOR WHEAT PROTEIN PERCENTAGES

	Swift Current	Scott	Lacombe	All Stations
S.E. (single determination)	.1503	.1559	.1387	.1485
S.E. (for mean of a variety)	.1063	.1102	.0982	.1050
N.D.* ($2 \times \sqrt{2} \times$ S.E. m)	.3007	.3117	.2777	.2969

* *Necessary difference for significance.*

Sampling or Plot Error

If the laboratory error were used as a basis for comparing varieties in their ability to produce high protein percentages it would at once be open to criticism, since there is no account taken of the variability occurring in the wheat from different plots. The analysis of variance was, therefore, calculated and the data secured are presented in Table II. The calculated necessary difference between the means of any two varieties at each of the three stations varied from 0.41 to 0.74 in protein percentage. The variances for varieties and for replications were removed from the total variance in calculating the necessary differences. The total variability, it will be noted, has been sub-

divided into three parts, namely, the varietal effect, the environmental effect attributed to replications, and that which remains, or not accounted for by either varieties or replications. It will also be observed from the F values that the variances for varieties and for replications at each of the stations were significant, demonstrating that real differences existed due to varietal effects as well as to environmental effects.

TABLE II

SAMPLING ERROR FOR DIFFERENCES BETWEEN VARIETIES AT EACH STATION

Variation due to	S.S.	D.F.	Variance	F	*5% Pt.	*1% Pt.
Swift Current						
Varieties	43.08	27	1.595	5.89	1.65	2.03
Replications	3.46	3	1.155	4.27	2.72	4.04
Remainder	21.92	81	.2706			
Scott						
Varieties	30.87	27	1.144	13.90	1.65	2.03
Replications	1.10	3	.366	4.45	2.72	4.04
Remainder	6.67	81	.0823			
Lacombe						
Varieties	75.35	27	2.791	27.84	1.65	2.03
Replications	1.32	3	.439	4.38	2.72	4.04
Remainder	8.13	81	.1003			

	Swift Current	Scott	Lacombe
Mean protein in %	15.31	15.97	13.96
S.E. (single determination)	.5202	.2869	.3167
S.E. (for mean of a variety)	.2601	.1435	.1583
N.D. ($2 \times \sqrt{2} \times$ S.E. m)	.7357	.4059	.4477

* *Approximate. Taken from Snedecor's tables (3).*

Application of the calculated necessary difference to the wheat protein percentages for each variety at each station (see Table IV) will indicate the varieties which are significantly higher than others in this characteristic at each station. To study the behavior of the varieties for the three stations combined, the calculations were extended to include all the data. In Table III it will be noted that significant values were obtained for varieties, replications, stations and for interaction of varieties and stations. The significant value for the interaction of varieties and stations indicates that the varieties did not behave exactly the same in relation to each other at all three stations. Since the necessary difference calculated from the interaction variance for varieties and stations is larger than the necessary difference calculated from the error variance for the three stations, then the larger error should be used to predict whether the differences observed are consistent for the three localities and are likely to be of the same order if the test were repeated.

TABLE III

SAMPLING ERROR FOR DIFFERENCES BETWEEN VARIETIES FOR THE THREE STATIONS

Variation due to	S.S.	D.F.	Variance	F	5% Pt.	1% Pt.
Varieties	115.92	27	4.29	29.2	1.56	1.87
Replications	5.88	9	.6532	4.4	1.96	2.56
Stations	236.52	2	118.26	804.7	3.04	4.70
Varieties × Stations	33.39	54	.6183	4.2	1.97	2.60
Remainder	35.71	243	.1470			

Necessary differences based on error variance	
S.E. for single determination	.3834
S.E. for mean of a variety	.1107
N.D. ($2 \times \sqrt{2} \times$ S.E. m)	.3131

Necessary differences based on variance for interactions of varieties and stations	
S.E. for single determination (4 plots)	.3934
S.E. for mean of a variety	.2270
N.D. ($2 \times \sqrt{2} \times$ S.E. m)	.6420

TABLE IV

MEAN PROTEIN PERCENTAGES FOR THE 28 VARIETIES TOGETHER WITH A CLASSIFICATION BASED ON NECESSARY DIFFERENCES

Variety	Mean for 3 Sta.		Swift Current		Scott		Lacombe	
	Protein, %	Class*	Protein, %	Class**	Protein, %	Class**	Protein, %	Class**
Reward 26–32	15.97	1	16.23	1	16.58	2	15.10	3
Reward Ott. 928	15.75	1	15.63	0	16.45	1	15.18	3
Reward 26–43	15.74	1	15.70	0	16.65	2	14.88	3
Reward Morden	15.74	1	15.90	1	16.28	1	15.05	3
Reward 28-25-1	15.73	1	15.80	0	16.20	1	14.95	3
944-A-33-11	15.69	1	16.05	1	16.75	3	14.25	0
Reward 22–42	15.67	1	15.95	1	16.28	1	14.78	2
Reward 22–35	15.62	1	15.65	0	16.28	1	14.93	3
Reward Long	15.61	1	15.83	1	16.18	0	14.83	2
Reward 3-25-A	15.56	1	15.65	0	16.30	1	14.70	2
Reward M × R R	15.55	1	15.70	0	16.08	0	14.88	3
944 A	15.46	0	16.05	1	16.50	1	13.83	0
Thatcher	15.14	0	15.75	0	16.28	1	13.40	−1
S.C. 26-264	15.10	0	15.13	0	15.90	0	14.28	0
G × P 2-27	14.96	0	15.45	0	16.08	0	13.35	−1
1325-29	14.91	0	14.78	−1	16.40	1	13.55	−1
Marquis	14.91	0	15.15	0	15.50	−1	14.08	0
M × P 1-27	14.82	0	15.03	0	15.75	0	13.68	−1
S.C. 26-268	14.81	0	15.05	0	15.50	−1	13.83	0
M × P 3-27	14.81	0	15.15	0	16.28	1	12.90	−3
M × P 7-27	14.76	0	15.20	0	16.00	0	13.08	−2
1319-6	14.69	0	15.13	0	15.68	0	13.28	−2
P × G 1-27	14.65	−1	15.25	0	15.75	0	12.95	−3
Garnet	14.31	−1	14.90	0	15.43	−1	12.60	−4
1320-23	14.29	−1	14.50	−1	15.38	−2	13.05	−2
Red Bobs 222	14.18	−1	14.15	−2	15.23	−2	13.15	−2
Shanks	14.07	−2	14.20	−2	14.60	−4	13.40	−1
1320-18	13.92	−2	13.65	−3	14.93	−3	12.93	−3
Mean	15.08		15.31		15.97		13.96	
N.D.		.46*		.54**		.29**		.32**

* *Based on variance for interaction between varieties and stations.*
** *Based on variance for error at the same station.*

In Table IV the 28 varieties are classified in respect to protein percentage for each of the three stations and for the mean values for the three stations combined. Each variety was tested against the mean protein of the remaining 27 by calculating the necessary differences from the formula $2\sqrt{\frac{n+1}{n}}\ s^2$ where $n = 27$ and $s =$ the standard error for a mean of a variety. It is interesting to note that the 10 strains of Reward were not significantly different in the averages of the three stations and all were higher than Marquis. It might be mentioned here that the Reward variety has for many years consistently given higher protein percentages than Marquis.

Individual Samples *versus* Composite Samples

In the testing of new varieties of hard red spring wheat for different areas of Western Canada it is customary to conduct protein determinations on samples grown in plots at a number of stations. Composite samples from the replicated plots grown at each station are used for this purpose rather than the wheat from each plot, owing to the multiplicity of tests which would be involved. Unless some knowledge of the necessary differences expected at each station is available, it is rather difficult to estimate a reasonable error for prediction although it would appear from this experiment that it would not be large. Since agronomists do not place much reliance on the results from any one station for one year it would appear that the use of composite samples would be justified, especially if the composite sample had essentially the same protein content as the mean protein for the replicated plots of each variety. If variety tests are conducted at a number of locations in a given area it would be a simple matter to calculate the variance remaining after removing variety and location variance from the total. The necessary differences calculated in this manner would correspond to that derived from the variance for interaction between varieties and stations and should, therefore, give a reasonable basis for prediction. Variety tests conducted at a number of stations would likely give a higher error for interaction effects than the error of the experiment in a properly planned system of plots.

The manner in which composite samples should be made up for quality tests has often been a question. Should equal quantities of wheat from each plot be combined to make up a composite or should an aliquot be taken from the thoroughly mixed samples from all the wheat produced by the four plots at each station? In order to obtain sufficient wheat for a milling test the latter procedure is often the only feasible one owing to low yields which might occur in one or more plots. In this experiment an opportunity to examine the two methods of sampling in respect to the protein determination was afforded. The protein percentages for the four plots for each variety at each station were averaged and this was compared with the calculated average protein percentages based on the grams of protein produced per plot. In other words, the yield in grams per plot was multiplied by the protein percentage to obtain the amount of protein in grams. The total number of

grams of protein for the four plots was divided by the total yield, thus giving the percentage protein for the variety, which should correspond to what would be expected if the protein were determined on the composite sample from all the wheat produced by the four plots. The data revealed that the two sets of protein percentages were almost identical and gave a correlation coefficient of $r_{xy} = 0.9998$.

Yield *versus* Protein Percentages

To determine the relation between yield and protein percentages the variances and covariances were calculated for each station from which the correlations were obtained, as given in Table V. The total correlations were subdivided into the various components to measure the effect of each on the relation of yield to protein content. The total correlations for the Swift Current and Scott stations were not large and that for the Lacombe station was insignificant. When the effect of varieties was removed from the total correlations, insignificant correlations between yield and protein percentage were obtained for each of the stations. The correlations for replications were not significant although a negative tendency was indicated. This is not surprising since only two degrees of freedom were available for testing the significance of the correlations. The residual variations in yield per plot were therefore not associated with the residual variations in protein percentage and this would account in large part for the close relation between the mean protein percentages for the four plots of a variety and the calculated protein percentages based on the grams of protein produced per plot.

TABLE V

CORRELATIONS FOR YIELD (y) AND PROTEIN (p) CONTENT OBTAINED FROM THE VARIANCES AND COVARIANCES FOR EACH STATION

	D.F.	$y\,p$
Swift Current		
Total	110	−.2959*
Varieties	26	−.5816*
Replications	2	−.7478
Total—varieties	83	−.0002
Remainder	80	.1605
Scott		
Total	110	−.2077**
Varieties	26	−.3713
Replications	2	−.4445
Total—varieties	83	.0829
Remainder	80	−.1532
Lacombe		
Total	110	.1304
Varieties	26	.2393
Replications	2	−.8147
Total—varieties	83	−.1902
Remainder	80	−.0705

* *Greater than* $P = .01$.
** *Greater than* $P = .05$.

TABLE VI

CORRELATIONS FOR YIELD (y) AND PROTEIN (p) CONTENT OBTAINED FROM THE VARIANCES AND COVARIANCES FOR THE THREE STATIONS COMBINED

	D.F.	yp
Total	334	−.7046*
Varieties	26	−.0945
Varieties × Stations	53	.1774
Total—varieties	307	−.8249*
Stations	1	−.9991**
Replications	8	−.6420*
Remainder	242	.0390

* *Greater than* $P = .01$.
** *Greater than* $P = .05$.

In Table VI the correlations are presented for the three stations combined. The major effects of environment, as represented by the correlations for stations and replications, indicate that these are chiefly responsible for the significant total negative correlation. The correlations for varieties and interaction of varieties and stations contribute very little to the total correlation. When all of these are removed from the total correlation, yield and protein content are not significantly correlated.

The Quality Testing of Wheat Varieties

The quantity of protein is an important factor in quality of wheat and also serves as a guide in interpreting baking test results. While this experiment deals only with the variability occurring in protein percentage in varietal trials, it suggests a reasonable basis of sampling for tests designed to determine the suitability or unsuitability, in quality, of varieties for certain areas. Variability in protein content within varieties at each station does not appear to be large if a well planned system of plots is used. To obtain a cross section of the quality behavior of varieties for a given area without entailing too many samples for testing, equal quantities of wheat for each variety from each point might be composited, thus pooling the environmental effects for that area. The manner of compositing, however, would depend on the soundness or suitability of the samples for quality tests. It would, of course, be advantageous to make a complete analysis for each variety grown at each location for a fuller interpretation of the adaptability of the variety to the area. A uniform series of varieties tested in several areas in this manner should help in the zonation of varieties for different areas. This procedure is receiving attention in Canada at the present time, as numerous plot tests of the better wheat varieties are being conducted in farmers' fields in conjunction with the experimental stations.

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PASTURE STUDIES XI.

PASTURE RESEARCH IN QUEBEC. CHEMICAL, ECOLOGICAL AND NUTRITIONAL PHASES[1]

By L. C. Raymond[2]

Abstract

Surveys of pasture flora in Quebec have led to the recognition of four layers, *viz.*, turf, tall herbaceous, shrub and tree. The human factor is advanced as the most important single agency determining the existing plant life but water supply and the fertility level exert important effects.

The soils under investigation carry sufficient seed to produce, on the average, 77 million potential plants per acre, but these seeds show a low correlation with the plants prevalent in the sward. It was revealed that each mature cow voids 1¼ million viable seeds per grazing season and that these seeds are closely related to the components of the sward.

Where phosphorus was applied as superphosphate at a rate of 700 lb. per acre, it was shown that most of that element was fixed in the top half-inch of soil and that the available portion of it was largely depleted after three crop years. Pot cultures of pasture soils growing either *Phleum pratense* or *Trifolium repens* show that calcium has depressed and sulphur has increased both the herbage yield and the uptake of phosphorus. The organic fraction of phosphorus in soils has been identified as containing 0.5% of lecithin and 65% of nucleic acid. The latter has been extracted quantitatively in pure form.

Mixed herbage and pure grass species have been fed to rabbits as a means of determining feeding value. The 35 groups fed have given highly variable results. Statistical examination of the data shows little if any correlation between gains and the constituents of the herbage as determined by a standard feeding-stuffs analysis. It has been tentatively concluded that the condition of the fibre, depending on the proportion of ligno and hemicellulose, is the most likely cause of the variability. The study is proceeding.

Introduction

Pasture research at Macdonald College had its inception in 1930. It very quickly became evident that there were involved a number of distinct phases which cut across ordinary departmental lines. As a result, a committee consisting of representatives of the departments of agronomy, animal nutrition, botany and chemistry was appointed in 1931, and has functioned since that time. Each member of the committee directs the work falling in his particular field.

The detailed features of the investigations are carried out by graduate assistants. At the present time three such men are appointed on a two-year basis, one in agronomy, one in animal nutrition and one in chemistry, with a part time botanical assistant. Each of the regular assistants gives full time in summer and half time during the winter months. This makes it possible for them to proceed to a higher degree in the two-year period. Some phase of the research work in progress is used as thesis material. The scheme has functioned very satisfactorily.

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Contribution from the Macdonald College Pasture Committee, Macdonald College, Quebec. Presented at the Ottawa Meeting of the Royal Society of Canada, May, 1936.

[2] *Chairman, Macdonald College Pasture Committee, and Assistant Professor of Agronomy, Macdonald College.*

Much of the research undertaken has already been published. This paper is a résumé of the more recent findings and of some of the work in progress. The various aspects will be grouped as far as possible for presentation.

I. Soil Zonation

At the outset, a very tentative and general grouping of the soils of Quebec was available (13). The main divisions recognized, at that time, were the Laurentian upland podsols, the Appalachian upland podsols, the brown forest soils and the marine, lake and river group. All of these are still in need of much clarification. Until 1935 the major part of the field work was confined to the brown forest area and, as time permitted, the dividing line between this group and the podsols to the south and east was studied in some detail. Fig. 1 shows the result of the surveys made. The region covered is divisible into four main zones, (i) the upland Appalachian heavily leached, podsolized soils; (ii) the less heavily leached brown forest soils, 400 feet or more above sea level; (iii) an area with the soils transitional between the two above; and (iv) the lowland types. The U.S. soil types adjoining the international boundary are also indicated.

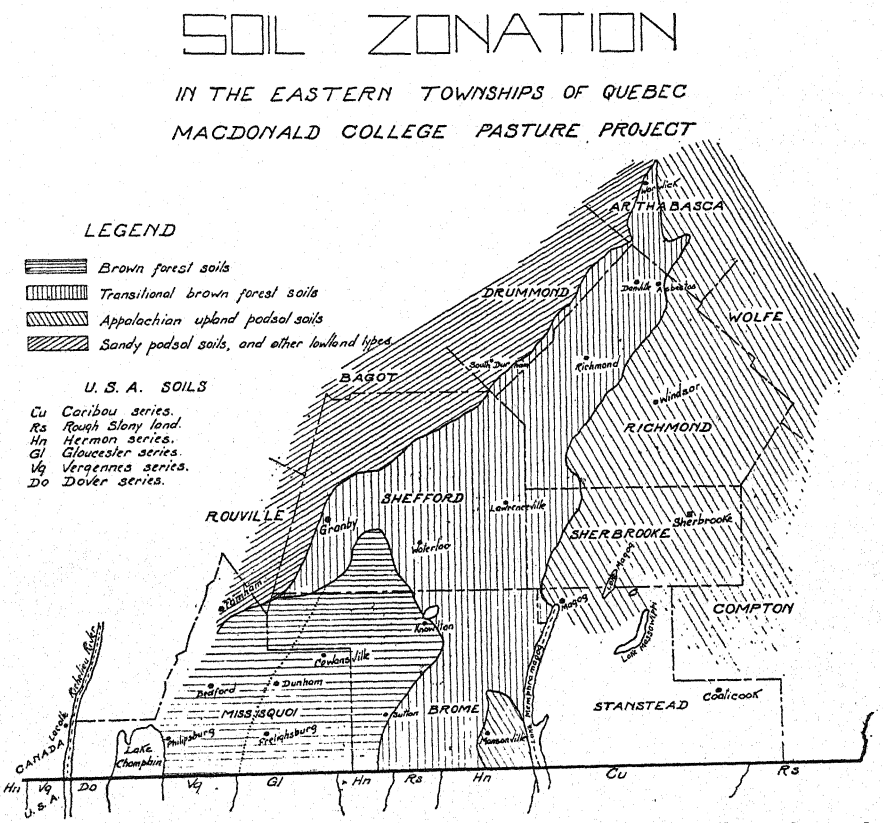


FIG. 1. *A section of the Eastern Townships of Quebec showing the soil zonation work accomplished to date.*

The nature of the two dominant soils in this locality has been quite fully discussed by McKibbin and Pugsley (12). The brown forest soils generally are deeper than the podsolized soils. In the former, organic and mineral matter are more intimately mingled, and the successive soil layers from the surface downward differ less markedly from one another than those of the podsols. In virgin podsols the surface few inches is very largely organic matter, with severely leached underlying material. Consequent upon their physical and chemical dissimilarity there are differences in biological response within and upon the soils of these zones.

II. Ecological Aspects

A. Survey

Following the soil classification the next logical step was to gain some knowledge of the existing flora. The first work of this nature was efforts by Newton and Nowosad (9) and Newton and Stobbe (10) to correlate vegetation with the various soil zones. They found, for example, that the brown forest soils were marked by deciduous woods composed chiefly of *Acer saccharum* mixed with *Fagus grandiflora*, *Betula lutea*, etc., and the podsols by mixed coniferous forest dominated in parts by *Picea canadensis*, *P. mariana*, and *Tsuga canadensis*, with *Acer saccharum*, *A. spicatum* and *A. pennsylvanicum* often present. They also listed some forest-floor plants and others of indicator value.

More recently, Whyte (16) has endeavored to make an ecological classification of the pastures, using as a basis of primary distinction the more conspicuous physiognomic features of the vegetation and working down to subdivisions requiring a rather detailed analysis of floristic composition. The more prominent pasture types can generally be related from mere observation to the environmental factors which tend to produce them, but the conditions which give rise to less distinctive communities are seldom obvious, and require for their elucidation more extensive research than is possible in a primary survey.

Most important in determining the physiognomic type is the human factor, acting both directly and through grazing animals. Without it, grassland would not even exist in this part of the world, and its mode of operation decides how far pasture shall replace the native woodland. Grazing tends to produce a turf of low-growing, rhizomatous herbs, with growing points protected by being held close to the ground. A period of undergrazing allows the establishment of taller herbs, shrubs and trees. Or again, incomplete destruction of the woodland species when the land was first cleared may result in the persistence of some of these in spite of grazing. Thus, as shown in Table I there is found very commonly in our pastures not only (i) the turf layer of vegetation but also taller layers which are alien to such a community. These may be subdivided into (ii) tall herbaceous layer, (iii) shrub layer, (iv) tree layer. Except for an occasional shade tree, these last three layers are undesirable, and owe their presence, as previously mentioned, to imperfect operation of the human and herbivor factors. The abundant presence of

TABLE I

SCHEME OF CLASSIFICATION OF PASTURE TYPES

Physiognomic type: prominent vegetation layer	Moisture relation	Layer	Dominant species in each layer on brown forest soils
I Turf	*Hydrophytic* high water table	I	*Cyperaceae; Agrostis stolonifera*
		II	*Eupatorium perfoliatum*
		III	*Salix; Alnus*
II Tall herb	*Mesophytic* medium condition	I	*Agrostis alba; Poa pratensis; Trifolium repens; Festuca rubra*
		II	*Solidago; Dicksonia punctilobula*
III Shrub		III	*Spiraea; Crataegus; Betula* scrub
		IV	*Acer saccharum; Fagus grandiflora; Betula lutea*
IV Tree	*Xerophytic* dry knolls and slopes	I	*Danthonia spicata; Poa compressa*

one or other tall layer affords, however, an easily recognizable basis of primary classification of pastures and one which also conveys some idea of their economic value.

Next to the human factor, that which has most effect on the type of vegetation is the water supply. The grassland formation is typically mesophytic, but where the water table is high a sward with *Cyperaceae* dominant affords grazing of some value. On the other hand, on knolls and steep slopes, the rapid run-off causes the ground to hold less than its share of summer rain and to support only a drought-enduring vegetation of poor feeding value, such as *Danthonia*, and deep-rooted herbs. This effect, as Johnstone-Wallace (8) has shown, is largely obviated if only a good spongy turf is developed on such places. The height of the water table and the water-holding capacity of the soil are, in fact, everywhere important because of the long spells of summer drought during which water is the limiting factor in the growth of grassland vegetation.

Consequently a secondary subdivision of pasture types is made on this basis.

Further subdivision is made in terms of floristic composition, using any or all of the layers that may be present. Newton and Nowosad (9) and Newton and Stobbe (10) found the tree species to be most characteristic of the genetic soil type. Whyte, whose survey so far has been mainly confined to the brown forest soil, has concentrated on the turf and intermediate layers. The former, of course, is the important one. The factors, apart from water supply, which determine the dominant species of the turf are still obscure but it is believed that the general fertility level is of major importance. This may depend on a variety of conditions, notably the degree of leaching and podsolization, of soil erosion, of soil exhaustion, etc. In the primary survey

work, the botanical composition of the sward is arrived at either by eye observation and listing of estimated frequency, or by point quadrat readings. Analyses of typical swards are given in Table II.

TABLE II

POINT QUADRAT METHOD—"HITS" PER HUNDRED POINTS EXAMINED

	1*	2	3	4	5
Poa compressa	—	—	—	11	—
Festuca rubra	41	3	—	7	—
Agrostis alba	8	12	30	23	7
Poa pratensis	10	26	7	24	45
Trifolium repens	6	12	9	15	11
Achillea Millefolium	5	1	10	4	4
Phleum pratense	3	—	10	1	2
Viola spp.	3	—	2	1	—
Panicum lanuginosum	1	—	—	—	1
Fragaria virginiana	1	1	2	—	—
Hieracium aurantiacum	1	1	3	16	—
Taraxacum officinale	1	—	—	—	—
Cerastium vulgatum	1	3	—	—	—
Chrysanthemum Leucanthemum	1	—	4	—	—
Prunella vulgaris	1	2	4	—	—
Veronica serpyllifolia	1	3	—	—	1
Moss	8	3	3	—	—
Bare ground	26	27	22	14	34
Plantago major	—	1	—	—	—
Oxalis europaea	—	2	1	—	—
Plantago lanceolata	—	1	—	—	—
Aster spp.	—	1	—	—	—
Solidago spp.	—	1	—	—	—
Festuca elatior	—	—	—	—	2
Rumex Acetosella	—	3	1	1	4
Danthonia spicata	—	14	4	—	—
Hieracium florentinum	—	—	3	—	—
Carex spp.	—	—	3	—	—
Ranunculus acris	—	—	—	2	—

*1 *Festuca sward; Lawrenceville, July 1935.*
2 *Poa : Danthonia, Agrostis, Trifolium; Dunham-Sutton Rd., June, 1935.*
3 *Agrostis sward; Dunham-Sutton Rd., June 1935.*
4 *Poa-Agrostis sward; Oak Hill, June, 1935.*
5 *Poa sward; Bull Pond, June, 1936.*

The sward which is most general in the pastures of the Eastern Townships is one in which *Agrostis* spp. are dominant. Under mesophytic conditions, *Agrostis alba* is the prominent species. Here there may be an almost pure *Agrostis* sward, or a combination with *Trifolium repens* or *Poa pratensis*, or both. Generally speaking, *Agrostis alba* is believed to be indicative of a lower fertility level than is required by *Poa pratensis*. This is borne out by the fact that in the podsolized brown forest soils, *Agrostis alba* is the dominant species, while *Poa pratensis* although present does not become dominant. On the other hand, in paddocks where the cows are confined for milking, and in night pastures where the ground has received more than its fair share of droppings, *Poa pratensis* is seen to flourish (7). This dominance of *Poa pratensis* linked with abundance of *Trifolium repens* marks the best type of

pasture to be found in this area. Where hydrophytes prevail, *Agrostis alba* is also present, but *A. stolonifera* is more abundant. *Festuca rubra* presents a more difficult problem because of its sporadic occurrence. It may occur in swards covering large areas or in locally dominant patches. It has been found in comparatively wet areas but on the whole it favors drier places. It is found associated with *Agrostis alba* on the one hand and with *Danthonia spicata* on the other, and because of its distribution and associations, is presumably indicative of a fertility somewhat lower than that required by *Agrostis alba*. Of the other grasses occurring, only one is to be found in any quantity, *viz.*, *Danthonia spicata*. This grass inhabits dry places and pastures that have run out, and, as its name "Poverty grass" implies, it is a sure sign of a very low level of fertility. It is not relished by stock and is indeed a weed in the worst sense of the word.

Besides pastures in which the turf layer is most abundant, two other types are of quite common occurrence, namely those which consist largely of hardhack *(Spiraea tomentosa)* or of hay-scented fern *(Dicksonia punctilobula)*. Spiraea pastures occur widely in dry and sometimes in wet land, and are probably the result of undergrazing. *Dicksonia punctilobula* is also widespread, probably as a relic of the woodland flora. It grows in clumps and, while in a hardhack pasture there is usually some feed round the bushes, where *Dicksonia* grows, grass flourishes only in the spaces between the clumps. *Dicksonia* is thus a bad weed.

A much more detailed study of the turf layer in particular has been made by Dore (6) who has made use of the experimental fertilizer plots, referred to later in this paper, to determine the succession of grasses and clovers that takes place when the fertility factor is varied.

B. Natural Re-seeding

The natural re-seeding habits of plants associated with pastures is another ecological phase that has been given some attention. Dore (5) discusses the methods by which the flora of the turf layer provide for their maintenance. Propagation by vegetative means is by far the most important in permanent pastures. Some plants, of which *Trifolium repens* is an excellent example, increase by stolons. Others again have the underground rootstock or rhizome which is characteristic of *Poa pratensis*, *Agrostis stolonifera* and many others. The second important method of propagation is by seed, which may be distributed by natural means or transported by animals. Most of the seeds scattered by animals must possess resistance to the digestive processes.

In studying this question Dore collected samples of both soil and manure from old permanent pastures. Sods 6 × 6 × 1 in. were lifted in June and September from four pastures, and fresh manure droppings were obtained in early, middle and late summer from two areas. The samples were dried, broken up, mixed and sampled. Standard quantities were placed in flats of sterilized soil in the greenhouse to germinate. A duplicate set was subjected to a previous freezing and thawing to more nearly simulate field conditions.

Table III gives a list of the species found growing on the four untreated pastures worked with, together with their relative frequency expressed as percentage of ground covered.

TABLE III

PERCENTAGE GROUND COVERED BY SPECIES OF PLANTS ON UNFERTILIZED AREAS OF THE FOUR PERMANENT PASTURES IN THE VICINITY OF COWANSVILLE, QUE.

Species	Pasture				Average
	A	B	C	D	
Agrostis stolonifera	20.6	10.6	20.3	24.6	19.3
Festuca rubra	–	35.6	2.1	1.4	9.8
Hieracium aurantiacum	12.4	1.7	14.4	+	7.2
Trifolium repens	5.3	10.9	.8	9.4	6.6
Phleum pratense	8.7	2.1	9.5	4.4	6.2
Taraxacum officinale	6.7	1.2	+	13.3	5.3
Poa pratensis	4.0	2.0	+	6.7	3.2
Danthonia spicata	+	3.6	8.1	+	3.0
Plantago major	.8	.6	1.3	5.6	2.1
Ranunculus acris	2.1	.8	.5	5.0	2.1
Prunella vulgaris	2.4	1.4	1.3	2.1	1.8
Panicum lanuginosum	1.7	+	4.8	+	1.7
Fragaria virginiana	1.6	1.8	2.6	+	1.5
Carex spp.	3.2	+	1.5	.6	1.4
Oxalis europaea	1.1	2.2	1.4	+	1.3
Chrysanthemum Leucanthemum	+	1.9	1.8	+	1.1
Potentilla simplex	–	+	4.0	+	1.1
Cyperus diandrus	+	–	–	3.9	1.0
Solidago spp.	+	+	1.7	+	.7
Viola pallens	1.1	.7	+	.6	.6
Digitaria Ischaemum	+	2.5	–	–	.6
Hieracium florentinum	1.3	+	.5	–	.5
Achillea Millefolium	+	1.7	+	+	.5
Juncus macer	.9	+	+	.7	.4
Antennaria neglecta	.7	.7	+	+	.4
Linum catharticum	–	–	–	1.4	
Poa compressa	.5	+	+	.6	
Glyceria striata	.5	–	–	+	
Stellaria graminea	+	–	.6	–	
Trifolium agrarium	–	+	.7	+	
Cerastium vulgatum	+	+	+	+	
Veronica serpyllifolia	+	+	+	+	
Cirsium spp.	+	–	+	+	
Hedeoma pulegioides	+	–	–	–	
Lycopus spp.	+	+	+	+	
Oenothera pumila	+	+	+	+	
Potentilla norvegica	+	+	+	+	
Sisyrinchium angustifolium	+	+	+	+	
Spiraea tomentosa	+	+	+	–	
Sporobolus neglectus	+	–	–	–	
Vicia Cracca	–	–	+	–	
Trifolium pratense	–	+	+	+	
Leontodon autumnalis	+	+	–	+	
Rumex Acetosella	+	+	+	+	
Hypericum spp.	+	+	+	+	
Festuca elatior	+	+	+	+	
Hydrocotyle americana	+	+	+	+	
Agropyron repens	+	–	–	–	
Equisetum arvense	+	–	–	+	
Echinochloa crusgalli	+	–	–	–	
Erigeron sp.	+	+	+	+	
Galium sp.	+	+	+	+	
Lobelia inflata	+	+	–	–	
Plantago lanceolata	–	+	–	–	
Veronica officinalis	–	–	+	–	
Setaria lutescens	–	–	–	–	

Plants present in less than 0.5% of ground cover are indicated by +.

The data obtained from the soil samples are presented in a very brief way in Table IV. This shows an average for the district of 77 million potential plants per acre, which represents more than four times the number of actual plants ordinarily found growing in a permanent pasture. The seeds were, for the most part, from plants that were not very important in the sward. Five species that contributed 63% of the seed represent less than 7% of the sward.

TABLE IV

THE MORE IMPORTANT SEEDLINGS ARISING FROM VIABLE SEEDS IN SOIL FROM PERMANENT PASTURES

Species	Number of seedlings per sq. ft. of pasture surface				Ave. no. per acre, millions
	A	B	C	D	
Cyperus diandrus	56	—	—	366	18.38
Juncus macer	88	15	97	85	12.41
Danthonia spicata	—	108	39	1	6.45
Chrysanthemum Leucanthemum	1	2	115	—	5.14
Cerastium vulgatum	16	—	28	51	4.15
Panicum lanuginosum	21	—	59	—	3.48
Agrostis stolonifera	28	7	24	10	3.01
Plantago major	14	1	2	40	2.48
Potentilla norvegica	6	16	19	15	2.44
Erigeron ramosus	—	—	54	1	2.40
Hypericum spp.	31	—	4	14	2.13
Digitaria Ischaemum	—	31	—	8	1.70
Lobelia inflata	9	9	1	12	1.35
Oenothera spp.	6	1	17	5	1.26
Poa compressa	13	4	—	7	1.05
Forty-four others in smaller amounts					
Total number of seedlings:	342	218	537	675	77.19
Number of species	32	21	33	32	57

The manure samples examined presented quite a different situation. From the standpoint of the species involved, livestock represents the most important seed distributing agency. Each mature cow was shown to distribute, on the average, more than one and one-quarter million seeds during a single grazing season. Table V gives a much curtailed picture of the results obtained. Five of the most valuable pasture plants contribute 70% of the seed. As would naturally be expected, a high correlation was found between the seasonal flora and seeds present in the manure samples.

Dore's data show an enormous potential supply of new plants available to the pasture sward. Under suitable conditions this supply may become a very potent factor. Results both here and elsewhere, however, leave little doubt that vegetative development is mainly responsible for the multiplication of the characteristic herbage species typical of a permanent pasture, when favorable conditions for that development are provided.

TABLE V

THE MORE IMPORTANT SEEDLINGS ARISING FROM VIABLE SEEDS IN MANURE FROM PERMANENT PASTURES AT PERIODS DURING THE SEASON

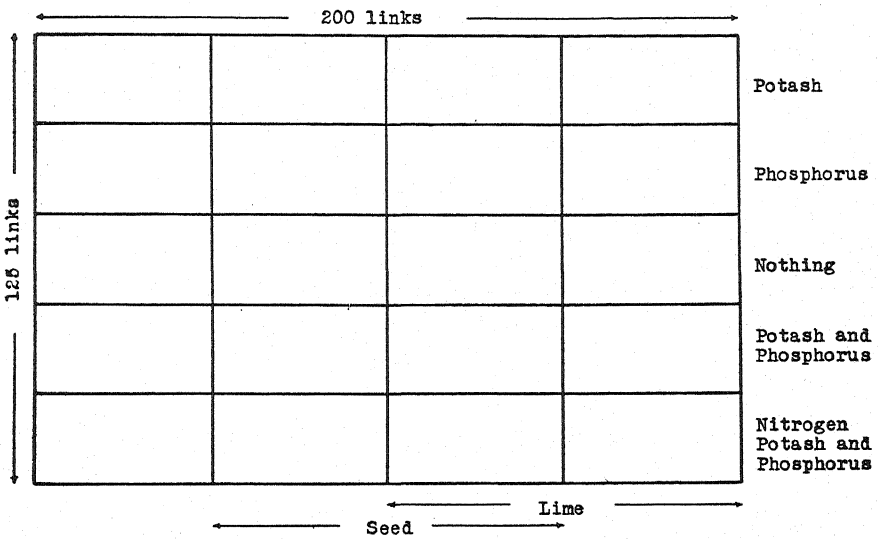
Species	Number of seedlings per 10 ounces of dried cattle manure						No. distributed by cow in 165 days, thousands
	Pasture B			Pasture D			
	June 20	Aug. 6	Sept. 10	July 20	Aug. 15	Sept. 10	
Agrostis stolonifera	—	237	118	259	91	44	291.34
Phleum pratense	18	39	347	25	47	55	206.54
Poa pratensis	1	199	12	132	17	29	151.69
Trifolium repens	3	161	25	67	42	19	123.30
Chenopodium album	—	—	245	—	—	6	97.63
Poa compressa	2	65	24	85	33	28	92.18
Cerastium vulgatum	65	15	2	7	5	—	36.56
Carex spp.	—	7	14	51	19	—	35.40
Plantago major	2	2	64	3	5	15	35.40
Veronica serpyllifolia	57	2	4	2	—	—	25.28
Danthonia spicata	5	11	15	17	4	—	20.23
Ranunculus acris	—	4	14	9	6	—	12.84
Plantago lanceolata	—	7	7	15	—	—	11.28
Thirty-six others in smaller amounts							
Total number of seedlings	167	795	970	719	291	212	1228.70
Number of species	13	26	33	27	19	19	49

III. Location and Experimental Procedure

Reference has already been made to the association of these investigations with the brown forest soil type. Representative farms were chosen centering about the town of Cowansville, Que. The initial procedure was concerned largely with the establishment of a satisfactory technique. While seeking possible means of improvement it was recognized that a typical pasture environment must be maintained. Seven farms were originally selected and surface applications of the standard fertilizers with and without lime and seed were given. Fig. 2 shows the arrangement of the first experimental areas. Weaknesses fairly soon became apparent in this method as the response to the mineral and complete fertilizers was so outstanding that the plots were almost immediately subjected to gross overgrazing. This difficulty was overcome in subsequent years by fertilizing from three to five acres surrounding the trial section and the plan of the experiments has been radically altered to measure as well the rates of the minerals. Randomization of the various treatments has also been resorted to. The standard plan at present in use is shown in Fig. 3.

Yields have been taken each year from the treated plots through the use of a relatively inexpensive wire cage which covers exactly a square yard. Clippings are made four times during the season, the cage being moved each time to a fresh area. The clipped samples are dried to constant weight with as little delay as possible.

Plan of Series I Pasture Plots



Rate of Fertilizer Application:

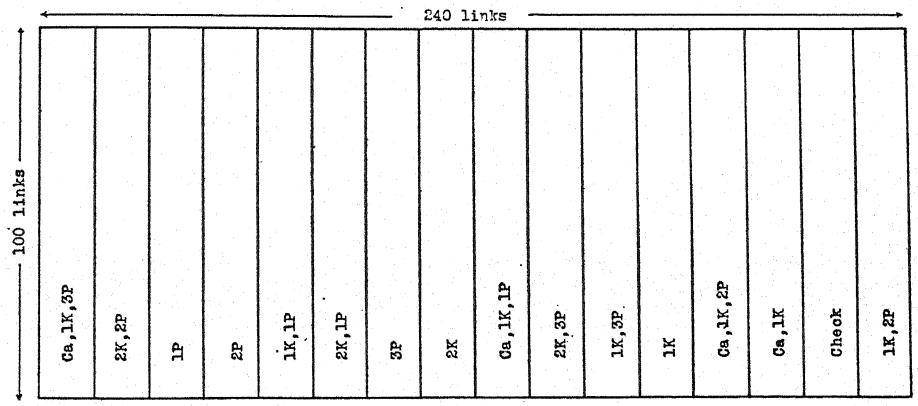
Nitrate of Soda	-	200 lbs.
Superphosphate 16%	-	500 "
Muriate of Potash	-	160 "
Ground Limestone	-	4000 "

Seed Mixture:

Kentucky Blue	Meadow Fescue
Canada Blue	Red Fescue
Red Top	Awnless Brome
Orchard Grass	White Clover

FIG. 2. *Plan of early plot experiments used in connection with this project.*

Plan of Series V Pasture Plots



Key to Treatments:

1P = 300 lbs.	Superphosphate 20%	
2P = 500 "	" "	"
3P = 700 "	" "	"
1K = 100 "	Muriate of Potash	
2K = 200 "	" "	"
Ca = 4000 "	Ground Limestone	

Systematic Arrangement of Plots:

1. Check	7. 1K,2P	13. CalK
2. 1P	8. 1K,3P	14. Ca,1K,1P
3. 2P	9. 2K	15. Ca,1K,2P
4. 3P	10. 2K,1P	16. Ca,1K,3P
5. 1K	11. 2K,2P	
6. 1K,1P	12. 2K,3P	

FIG. 3. *The standard type of plot experiment now employed.*

Careful record has been made of the floral change resulting from the treatments given. The grid quadrat was used for this purpose, the results being recorded as percentage of ground covered by the various species. The standard size now in use is 50 × 50 cm. divided into the usual 25 squares of 10 cm.

Inasmuch as a great many of these data have already been published (6, 11) no detailed reference will be made to yield or succession data in this paper.

IV. Chemical Phases—Phosphorus Studies

During the past five years yield data have been collected from a large number of plots located on representative brown forest soils. The results obtained on these soils show that, among the elements applied as chemical fertilizers and amendments, phosphorus is outstandingly the most significant in increasing the yields and quality of pasture herbage. Table VI A, from Stobbe (15), shows the effect of phosphate fertilization on the yield of the clipped herbage. The B. section of the Table records the effect of phosphorus on the nitrogen (N_2) content. From the succession studies it is apparent that much of this increase is an indirect one resulting from the material increase in the clover fraction of the clippings. The effect on the phosphorus content of the clipped herbage due to applications of superphosphate is shown in the C. portion of Table VI. This again shows a marked increase in the phosphorus (P) on plots that were treated with that element. This result probably is indirect to some extent as well.

The highly significant action of phosphorus has led to a study of its soil and plant relationships.

TABLE VI

A. EFFECT OF PHOSPHATE FERTILIZATION ON THE YIELD OF HERBAGE CLIPPED FROM PLOTS, 1931
(6 farms, 24 plots per treatment)

Treatments with P	Total weight of clipping	Treatments without P	Total weight of clipping
CaPK	2642	CaK	1767
PK	2427	K	1473
CaP	2302	Ca	1611
P	1966	Nil	1425

B. EFFECT OF PHOSPHATE FERTILIZATION ON THE NITROGEN CONTENT OF HERBAGE CLIPPED FROM PLOTS, 1931
(6 farms, 24 plots per treatment)

Treatments with P	Average % N_2 in herbage	Treatments without P	Average % N_2 in herbage
CaPK	3.44	CaK	2.74
PK	3.37	K	2.56
CaP	3.14	Ca	2.53
P	2.87	Nil	2.52

C. EFFECT OF PHOSPHATE FERTILIZATION ON THE PHOSPHORUS CONTENT OF HERBAGE CLIPPED FROM PLOTS, 1932
(6 farms, 24 plots per treatment)

Treatments with P	Average % P in herbage	Treatments without P	Average % P in herbage
CaPK	0.338	CaK	0.279
PK	0.334	K	0.282
CaP	0.327	Ca	0.292
P	0.345	Nil	0.295

A. Method of Extracting Available Soil Phosphates

The currently used methods of extracting soil phosphates for study have not been conceived primarily from the standpoint of soil conditions. Russell (14) shows that the soil drainage water, obtained through drainage, not run-off, contains a high proportion of calcium among cations, and sulphate among anions. Analytical figures for the water solutions from North American soils show similar high proportions of lime and sulphate sulphur in the total solids. Wrenshall and McKibbin (17) have employed a solution, the use of which is termed the "Quebec" method, for the extraction of phosphate phosphorus in which calcium is the dominant cation and sulphate the dominant anion. The solution is made up to have a reaction value of pH 3.0. Results are given showing that, by this method, the extracted phosphates conform more closely to the plant growth response.

B. Penetration of Applied Phosphorus

Samples of permanent pasture soils both treated and untreated have been collected and extracted to determine the vertical distribution of the phosphate phosphorus which is available to plants. The samples taken were the upper half-inch, the next inch and the next one and one-half inch. Treatment consisted of 700 lb. of 16% superphosphate applied as a surface dressing. The soils were sampled approximately four months after the treatment was given. Table VII shows the amount and location of the total and available phosphates under the two conditions. Much the greater part of the applied phosphorus is retained in the upper half-inch of the soil.

TABLE VII

VERTICAL DISTRIBUTION OF PHOSPHORUS IN FERTILIZED AND UNFERTILIZED BROWN FOREST PASTURE SOIL

Depth of soil sample	p.p.m. of P in air dry soil		
	Unfertilized		Fertilized
0 – ½ in.	Total	1000	1200
	Available	12	80
½ – 1½ in.	Total	850	880
	Available	8	8
1½ – 3 in.	Total	700	700
	Available	7	7

C. Depletion of Phosphorus by Cropping

In Table VIII data are presented showing the available phosphate phosphorus of a fertilized soil one, two, and three years after application of 500 lb. per acre of 16% superphosphate to the surface.

TABLE VIII

DEPLETION OF AVAILABLE PHOSPHORUS FROM SURFACE HALF-INCH OF BROWN FOREST PASTURE SOIL

Designation of sample	Year of fertilization	No. crops removed since fertilization	Available P in p.p.m. of soil at Sept. 1934
D_4PA	1934	1	53
D_3PA	1933	2	33
D_2PA	1932	3	12
D_2NA	–	–	6

It will be noted that the available phosphorus rapidly decreases annually, and after three years closely approaches the original content.

D. Pot Cultures with Brown Forest Soils

In order to study further, under close control, the response of pasture plants to soil conditions, 78 pot cultures were established in the greenhouse. The soil used was a typical brown forest soil under permanent pasture. The two indicator plants chosen were wild white clover and timothy, and each of them was propagated clonally for this trial. The former in particular has, in the field, given very striking response to phosphate fertilization in Quebec, New York state and in the British Isles. The experiment is not yet complete but in Table IX results are presented for the first two clippings illustrating the effects of lime and sulphur applied to the soil alone and with added phosphate phosphorus.

TABLE IX

A. TOTAL YIELDS OF TIMOTHY AND CLOVER TAKEN FROM TREATMENTS IN FIRST TWO CLIPPINGS
(Pot cultures)

Treatment, pounds per acre		Yield in grams
Nil		11.0
Limestone	2000	9.0
Sulphur	150	13.1
Superphosphate	300	13.8
Superphosphate + limestone	300 + 2000	12.0
Superphosphate + sulphur	300 + 150	14.5
Superphosphate	700	15.7
Superphosphate + limestone	700 + 2000	15.0
Superphosphate + sulphur	700 + 150	18.4

B. PHOSPHORUS UPTAKE FROM TREATMENTS BY GROWING TIMOTHY AND CLOVER
(Pot cultures)

Treatment, pounds per acre		Milligrams of P removed by plants
Nil		11.5
Limestone	2000	9.5
Sulphur	150	15.8
Superphosphate	300	19.2
Superphosphate + limestone	300 + 2000	14.7
Superphosphate + sulphur	300 + 150	18.9
Superphosphate	700	24.2
Superphosphate + limestone	700 + 2000	20.8
Superphosphate + sulphur	700 + 150	26.7

It will be seen that lime, which lessens soil acidity, exerts a repressive effect on phosphorus uptake by plants and on the dry matter in crop yields, while elemental sulphur, which increases soil acidity, has increased both phosphorus uptake by plants and crop yields in dry matter. It is interesting to note that these facts coincide with observed, but unpublished, field results obtained in Quebec with lime and sulphur on pasture soils of a similar character. The clover and timothy independently show these responses.

E. Nature of Organic Phosphorus in Soils.

Phosphorus in Quebec pasture soils is usually present in considerable quantity, but not, however, in a form readily available to plants. The nature of soil inorganic phosphorus is fairly well known. A laboratory study is being made of the molecular forms in which this element in organic combination exists in our soils. This investigation is still in progress but is sufficiently advanced to permit the presentation of some results relevant

to the organic fraction. Two soils have been worked with, a typical brown forest pasture soil and a black muck soil, high in organic matter. In the former, 30% of the phosphorus was in the organic form and 70% inorganic, while in the muck soil 55% was organic and 45% inorganic. The most complete study, to date, has been made of the black muck soil. It was high in phosphorus to begin with, having a total phosphorus (P_2O_5) content of 0.5%. Of the total organic phosphorus present less than 0.5% was found to be lecithin and somewhat more than 65% was nucleic acids. The remaining portion has not yet been identified but may also be, at least in part, nucleic acids. The method of isolation and purification of soil nucleic acids has been revised and improved.

V. Nutritional Studies

In 1930 a series of three grazing trials was outlined, two trials with dairy cattle and one with beef steers, with the object of measuring the effects of mineral fertilization of natural pastures on their stock carrying capacity. The dairy cattle trials were discontinued after the first year, but the steer grazing tests have been continued. The results of the first four years of this project (2) indicated a very marked increase in steer carrying capacity resulting from the treatments given. Only one season's results are thus far available from the test now in progress. These show an increase of some 57% in total weight of beef produced per acre and 75% in steer days of grazing creditable to fertilization.

It was felt, however, that while grazing trials gave certain useful information, they could not be expected satisfactorily to measure the nutritive value as distinct from yield of pasture herbage. It was also felt that the nutritive value or feeding value per unit of weight of feed might in many cases be of as much importance in explaining the results of feeding tests as yield of herbage.

In 1933 it was decided to undertake studies aimed at the evaluation of the nutritive value of pasture herbage through the controlled feeding of clippings. Any such plan automatically eliminated the possibility of using the larger farm animals in the feeding tests. The large quantities of clippings which would be required for such stock could not be arranged for under our conditions. Thus some laboratory animal seemed the only solution, and rabbits, because of the similarity of their natural diets to those of farm ruminants, were chosen. No data were available to us as to the technique of laboratory management of rabbits for tests in which feed consumption records were essential, nor were specifications of suitable hutches or feeding equipment to be had. The first step in the project was therefore one largely of technique, which took the most of our first season's feeding work. Our present equipment is very satisfactory for feeding and metabolism tests and, with normal diets, feed loss is reduced to something under 1% of the total allowance, which represents a very small experimental error as compared with other sources of error in such tests with any class of stock.

Preparation of Diets

The procedure followed in the collection and preparation of the diets has been practically the same throughout the work thus far. For the mixed herbage from actual pasture fields, the plan has been as follows: A reasonably smooth area in a pasture field is selected and fenced, and about one-third of it is treated with mineral fertilizer at the rate of 500 lb. superphosphate (16% P_2O_5) and 100 lb. muriate of potash per acre. This is done in the fall where possible. The next pasture season the areas are periodically mowed with a lawn mower, the aim being to clip the herbage frequently enough to keep it in about the stage of maturity that is found in grazed pasture. This usually means a clipping once a month, or perhaps oftener in the spring. In general the herbage will be cut when less than four inches in height. These clippings are dried in the sun, coarsely ground, and stored in metal containers for feeding.

In addition to the clippings from actual pastures, feeding tests have been made on samples of pure species of different grasses grown on plots of the Agronomy Department. These have been treated in the same manner as described for the mixed herbage clippings.

It might be stated here that it is recognized that herbage clippings cannot be considered to represent in every respect the material eaten by grazing animals. Selective grazing is not imitated by this method, and while in good pastures this feature may not be an important factor, it becomes increasingly of significance in cases where the mixed herbage contains appreciable amounts of material avoided by the animal. Further, sun drying in all probability results in the leaching of some nutrients and possibly in alteration or destruction of others (vitamins). This latter problem is not as serious within a trial as might be thought, since all diets have received the same preparation, and hence may not seriously complicate comparisons. In this respect it may be pointed out that the chemical analyses of the diets fed over the past three years fail to show changes indicating any marked effects traceable to this method of preparation which would interfere with comparisons made.

In trials at present under way, clippings are being taken from areas containing almost no other grasses than bluegrass and redtop and these are being artificially dried as they are clipped. Thus the problems presented by selective grazing, in so far as it concerns weeds and objectionable grasses, and by the changes from sun drying and exposure, are minimized.

Source of Rabbits

Excepting for one test, the rabbits used have been bred and raised at Macdonald College. The breeding does trace to two white segregates of the New Zealand Red breed purchased in 1930. The females now in the colony are either half or full sisters or mother and daughter in relationship. Test rabbits in any one season are all by the same buck. This breeding plan has given satisfactory uniformity in the test stock. In general the litters have been weaned at about seven weeks of age and the young rabbits put on test

rations within the next two or three weeks. The tests have been standardized to a seven-day preliminary period followed by a 28 day test period. For the most part the feeding groups in any one test have contained five rabbits.

Results of Feeding Tests

To review in detail the 35 standard feeding groups together with the numerous special studies which have been completed since this work was started is out of the question in this paper. Some of the findings have already been published (3, 4), while more recent ones have been summarized by Cameron (1).

Taking the data of all the standard tests over the three years one feature stands out above all others, namely that results (gains of rabbits) have not been duplicated from one year to another, though replicate tests using the same diets have yielded comparable findings. In particular it has been evident that the herbage of different years has shown markedly different levels of nutritive worth. In view of the widely different conditions of moisture and temperature during the three years involved, this would perhaps not be surprising, were it not for the fact that the chemical composition (usual feeding stuffs analysis) of the herbage samples has shown relatively little variation, as compared with that of the gains made.

Some idea of the variability between lots may be had from Table X. Lot averages for the 35 tests were used as single observations as the basis for these calculations.

TABLE X

STANDARD DEVIATIONS OF LOT MEANS

Variable	*n*	Mean	Standard deviation	Coefficient of variation
Gain—28 days	35	94.9143	198.06	208.67
Daily feed	35	110.7143	20.57	18.6
Initial weight of rabbit	35	1,366.3428	178.25	13.1
% Crude protein	35	15.8629	3.08	19.4
% Crude fibre	35	23.1429	2.46	10.6
% N-free extract	35	49.4286	3.33	6.7

It may be pointed out that the chemical data represent the diets as fed and hence include cases in which supplements such as casein or sugar were involved. These often altered the protein or fibre levels of the rations. Hence the variability of the protein and fibre values is larger than that for the herbage clippings alone.

Simple correlation studies of the data indicate no significant relations between gains and any other of these variables excepting fibre. Here the relation is negative ($r = -.5039$).

An analysis by the method of partial regressions in which the dependent variable was 28-day gain and the independent variables were daily feed, initial

weight of rabbits, percentage of protein, percentage of fibre, and percentage extract, shows very clearly that percentage fibre is the most important of N-free these factors influencing gains of the rabbits, and percentage of protein the least. Taken together these five variables account for but 38.4% of the variability of the gains, which of course means that other important factors are involved. In this connection an earlier report (3) suggested quality of protein as one possible factor. In any case it seems certain that quantity of protein was not a factor in these tests.

Percentage of fibre in the diet appears to be an item of some significance. It is entirely probable, however, that the nature of the fibre is of greater importance than the total amount. This is suggested by Woodman (18) and is further indicated in these data by the fact that the lots which show the greatest gain are those (i) in which the fibre has been reduced by dilution of the diet with a fibreless material such as sugar, or (ii) in which wild white clover was the herbage and in which a particularly small amount of stemmy material was present. Delete these cases and the correlation between amount of fibre and gain drops below significance.

The chief conclusion to which these data point is that the ordinary feeding stuffs analysis may be of questionable value in predicting the nutritive value of pasture herbage. This is especially true with percentage of crude protein and suggests that care should be taken in pointing to changes in protein level of the herbage as justification for fertilization or other pasture treatment.

Acknowledgments

The author wishes to acknowledge the assistance received in the preparation of this paper, from Dr. G. W. Scarth and Messrs. W. G. Dore and J. H. Whyte in the ecological aspects of the work, from Dr. R. R. McKibbin in the soil and chemical phases, and from Prof. E. W. Crampton in the nutrition work.

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VIRUS STUDIES

I. THE PRODUCTION OF ANTISERA IN CHICKENS BY INOCULATION WITH POTATO X[1]

By W. Newton[2] and H. I. Edwards[3]

Abstract

Chicken antiserum was produced by three wing vein inoculations with sap from *Datura meteloides* and *Datura Stramonium* plants infected with "potato virus X". Before injection, the saps were purified by the Bawden and Pirie method. This antiserum formed a conspicuous precipitate when incubated for three hours at 37° C. with similarly purified sap of these two plant species when they were infected with the X or healthy potato virus, but failed to form any precipitate when incubated in the same way with purified sap from virus-free plants. Two unknown viruses, one from spinach and the other from tomato were established as belonging to the X group by the precipitin reaction through the use of chicken antisera. The serological grouping was supported by the fact that the unknowns had similar, if not identical lethal temperatures, longevities in vitro, and host ranges as the ordinary potato virus X.

Introduction

The specificity of the antisera secured from rabbits inoculated with the sap of virus-infected plants has assisted materially in the classification of plant viruses. In the case of plants infected with the ordinary tobacco mosaic virus, the formation of precipitate through the union of sap and antiserum is independent of the plant host from which the virus-infected sap is derived, at least within the family *Solanaceae*, according to Beale (3, 4), Birkeland (5), and Chester (6). If the precipitin reaction is independent of the host, as all evidence to date seems to indicate, then serological methods will remove much of the confusion that has arisen in the classification of plant viruses through the existence of the same virus or strain of the same virus in distinct hosts. All investigators agree that two or more viruses may be quite distinct as far as symptom expression in specific hosts is concerned, yet may be serologically identical; hence the precipitin reaction will serve to establish fundamental groups, but other means may have to be employed to identify virus strains within a serological group.

Up to the present rabbits appear to have been used almost exclusively in the study of plant viruses. It may be of interest that chickens respond in a similar way to injections of purified plant saps infected with a specific virus.

Experimental

The saps from *Datura meteloides* and *Datura Stramonium*, respectively, infected with potato X, the healthy potato virus, were purified according to the method described by Bawden and Pirie (1). Three wing vein injections of 1.5 cc., 2 cc., and 2 cc. respectively were made at three day intervals into

[1] *Manuscript received September 28, 1936.*
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mature White Wyandotte chickens. Eight days after the last inoculation the blood samples were drawn. The serum from these blood samples produced conspicuous precipitates when incubated for three hours at 37° C. and cooled overnight at 7° C. with the saps of X-infected plants but produced no precipitates when incubated with saps similarly purified, but free from X virus infection. Two tobacco ring spot viruses, one originally isolated from spinach and the other from tomato, reacted positively with chicken antisera. Although these viruses proved to be distinct from each other and from the strains of the X virus so far isolated by us from potatoes, as judged by their symptom expression on several hosts, the fundamental properties of all three viruses, such as lethal temperature, longevity *in vitro*, and host range, were very similar if not identical. The precipitin reaction with chicken antisera has established them as belonging to the X virus group.

TABLE I

PRECIPITIN REACTION OF CHICKEN ANTISERA AND PURIFIED PLANT SAPS

Sap from	Virus present	Dilution of antigen (purified sap)			
		1 : 1	1 : 10	1 : 20	1 : 200
Datura meteloides	"X"	++++	+++	++	±
Datura meteloides	None	0	0	0	0
Datura Stramonium	"X"	++++	+++	++	±
Datura Stramonium	None	0	0	0	0
Nicotiana tabacum (White Burley)	Unknown virus from tomatoes	++++	+++	++	±
Datura Stramonium	Unknown virus from spinach	++++	+++	++	±

Discussion

Only two chickens provided the antisera used in these experiments but samples from both reacted specifically with three forms of the X virus; at least no visible precipitate could be seen when similarly purified but virus-free saps were incubated with either sample of antisera. The failure to detect any differences in the antisera reaction between different forms of the X virus and the entirely negative results with virus-free saps are in agreement with those of Bawden (2) who studied the antigenic properties of the X virus by means of rabbit antisera. It would appear that the antigen prepared by us from virus infected plants cannot be diluted to the same extent as the antigen used by Chester (7) against rabbit antisera. As will be seen from Table I, when the virus antigen was diluted to 1 : 200 the amount of the precipitate formed was so slight that it was barely detectable. A comparative study of chicken and rabbit antisera will be reported later.

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VIRUS STUDIES

II. STREAK X, A DISEASE OF TOMATOES CAUSED BY A VIRUS OF THE POTATO X GROUP UNASSOCIATED WITH TOBACCO MOSAIC[1]

By William Newton[2]

Abstract

A streak disease of tomatoes was found to be caused by a virus of the potato X group unassociated with tobacco virus 1. The disease markedly reduced the yield of marketable fruit in several greenhouses near Victoria. The symptoms resemble those induced by ordinary potato virus X in conjunction with tobacco mosaic. The host range, lethal temperature, longevity *in vitro*, and dilution extinction point of the virus resemble ordinary potato X. Streak X may be distinguished from ordinary potato X by the more pronounced symptoms it induces on tobacco, *Datura*, *Nicotiana glutinosa*, and tomato, and particularly by the streaking and necrosis of the stems and leaves of tomato. The virus causing this streak disease could not be recovered from Irish Cobbler potatoes after an incubation period of ten days, neither did the characteristic symptoms occur on tomatoes already infected with the ordinary potato virus X. The virus was recovered unchanged from X-free potato seedlings. The antigen reaction also proved that the streak virus belonged to the potato virus X group.

Introduction

A disease of tomatoes caused by a virus of the potato X group unassociated with tobacco mosaic was found in several greenhouses near the city of Victoria, British Columbia. A pronounced striping and necrosis of the stems and leaves and a blotching of the fruit were the characteristic symptoms. In several houses more than 50% of the plants were infected, and from the infected plants, little marketable fruit was secured. The characteristic symptoms resemble those of so-called "experimental streak", the disease which appears when tomato plants that are infected with tobacco virus 1 are re-inoculated with the potato virus X.

Experimental

Streak X was transmitted to tobacco (White Burley), *Datura Stramonium*, *D. meteloides*, *Nicotiana glutinosa*, and tomatoes by rubbing the leaf surfaces with a glass spatula moistened with the sap from infected tomato foliage. The information with respect to symptom expression is summarized in Table I

TABLE I

The symptoms induced by streak X and potato X on specific hosts

Virus	Tobacco (White Burley)	*Datura meteloides*	*Datura Stramonium*	*Nicotiana glutinosa*	Petunia	Tomato
Streak X	RMn	rMn	rMN	MN	0	MNS
Potato X	rm	rmn	m	m	0	m (faint)

Explanation of symbols: r = *rings*, m = *mottle*, n = *necrosis*, s = *streaks, and* 0 = *no visible symptoms. Capitalization indicates that the symptom is very pronounced.*

[1] *Manuscript received September 28, 1936.*
Contribution No. 464 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
[2] *Pathologist-in-charge, Dominion Laboratory of Plant Pathology, Saanichton, B.C.*

and is contrasted with the symptoms induced by a local form of the potato virus X isolated by inoculating *Datura Stramonium* with the sap of locally grown Irish Cobbler potato tops. It will be noted that the host range is similar, but in all cases the symptoms induced by streak X are more severe than those induced by potato X from Irish Cobbler potatoes. On tobacco plants inoculated with streak X, conspicuous multiple rings appeared as both primary and secondary symptoms. However, the secondary symptoms were more frequently a net-like mottle followed by considerable necrosis. Potato X on tobacco sometimes appeared as faint rings but more frequently as a mild mottle only. On *Datura Stramonium* the mottle induced by streak X was rapidly followed by necrotic lesions. Under the same conditions, potato X induced a mottle only. On *Datura meteloides*, the symptom expressions of the two viruses were practically identical. Those induced by streak X appeared in a shorter time and were slightly more pronounced. On *N. glutinosa*, the mottle was followed by a pronounced necrosis in the case of streak X but a mottle only appeared when this species was inoculated with potato X. On tomatoes inoculated with streak X, a blotchy mottle first appeared, followed by streaks of a purplish tinge on the leaves and broad streaks of a similar color on the stems. These streaks later became necrotic and brown. Potato X induced on tomatoes a faint and barely detectable mottle only.

The absence of the characteristic local lesions of tobacco virus 1 on *D. Stramonium* and *N. glutinosa* proved that this disease was distinct from any form of the "streak group" described by Ainsworth (1).

No significant difference was found in the lethal temperature, longevity *in vitro* or dilution extinction point of streak X compared with potato X, as may be seen by examining the data in Tables II, III, and IV. Their similarity is suggestive that the virus causing the tomato streak X belongs to the potato virus X group. The lethal temperature of the virus causing this disease is between 65° and 70° C., hence tobacco virus 1 is not involved in this disease as in the case of "experimental streak."

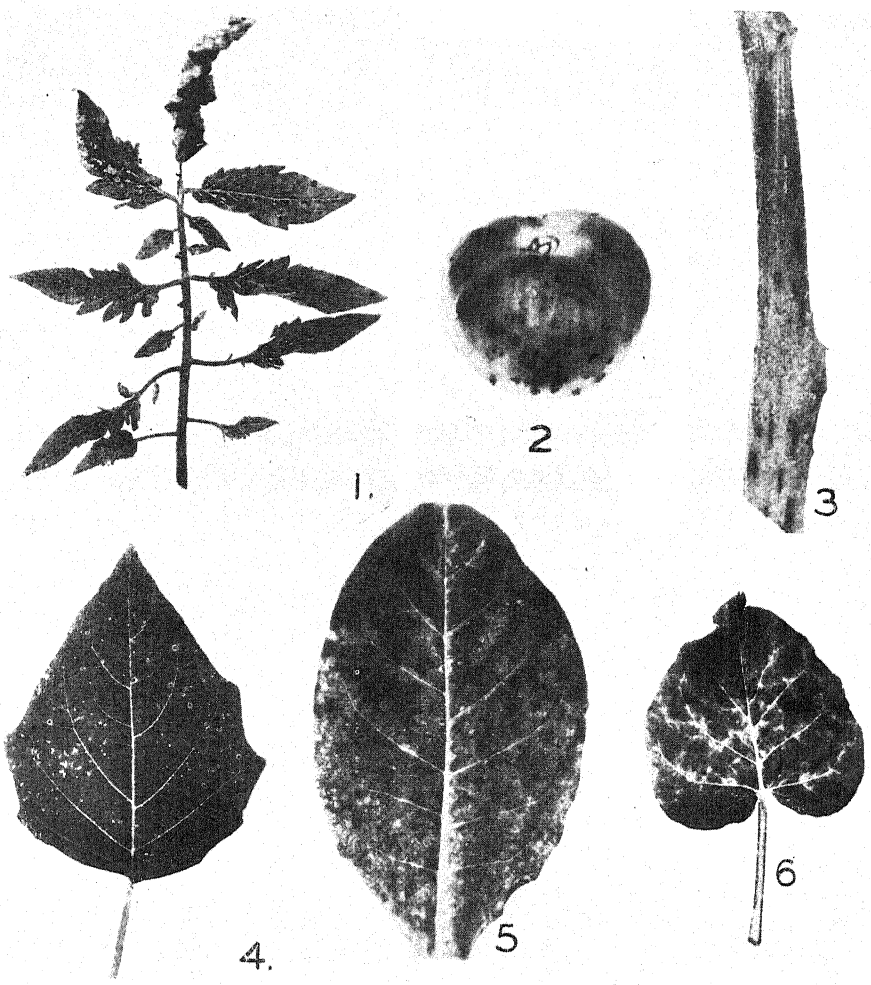
TABLE II

THE LETHAL TEMPERATURES OF STREAK X AND POTATO X IN *D. Stramonium* SAP DILUTED 1 : 20

Virus	Exposed in thin walled tubes for 10 min.				
	60°	65°	67°	69°	71°C.
Streak X	15*/15	15/15	15/12	15/2	10/0
Potato X (Irish Cobbler)	15/15	15/15	15/11	15/0	10/0

* *Number of plants inoculated over the number that became infected.*

PLATE I



Symptoms of streak X on tomato. (1) Leaf mottle and necrosis. (2) Fruit blotch. (3) Stem streaks. (4) D. meteloides, mottle and necrotic spots. (5) On tobacco, multiple rings. (6) On N. glutinosa, pronounced mottle.

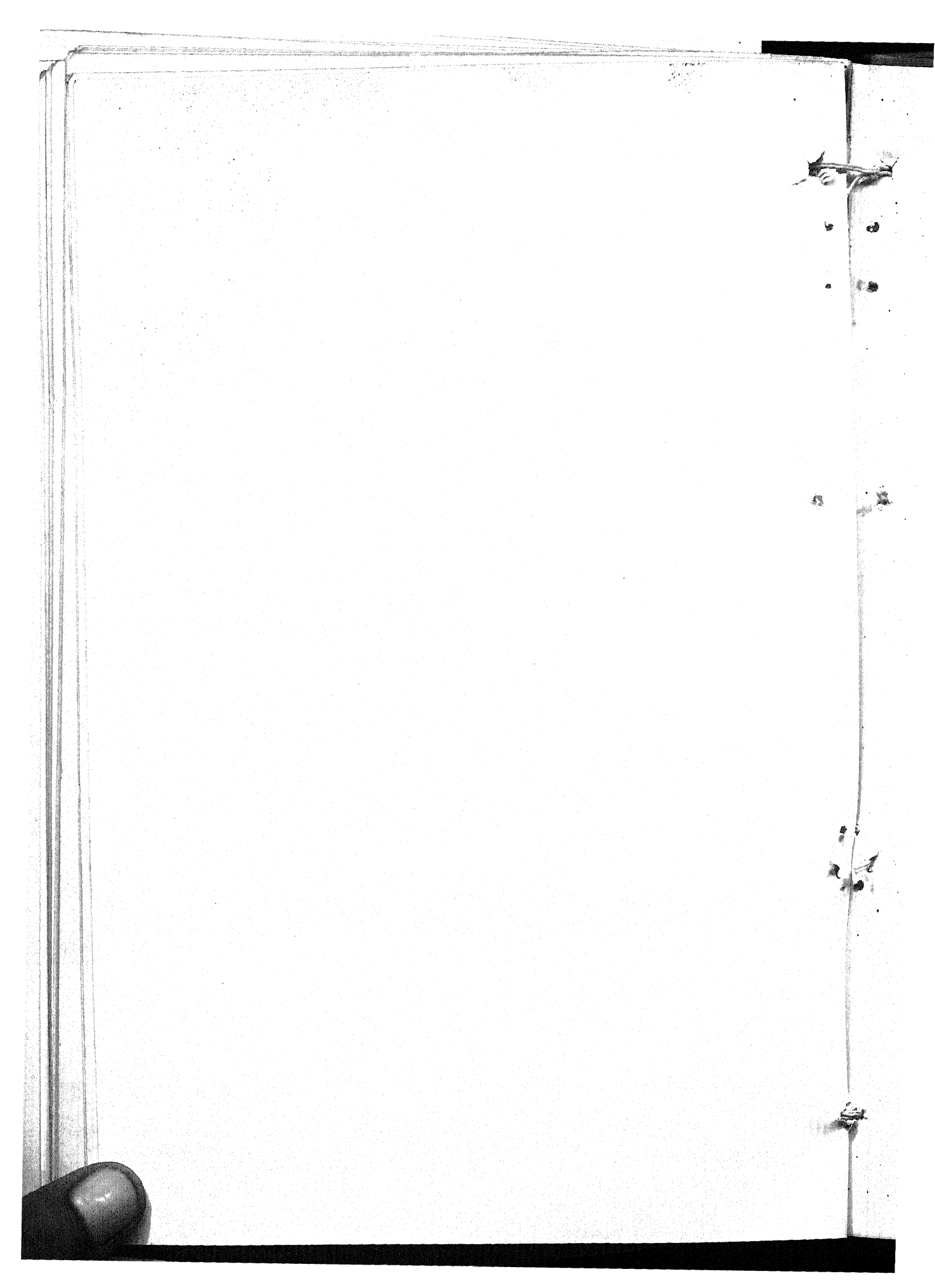


TABLE III

THE LONGEVITY OF STREAK X AND POTATO X IN *D. Stramonium* SAP DILUTED 1 : 100 AT ROOM TEMPERATURE (APPROXIMATELY 20° C.)

Virus	Days					
	1	4	8	12	16	20
Streak X	5*/5	5/5	5/5	5/5	5/2	5/0
Potato X (Irish Cobbler)	5/5	5/5	5/5	5/5	5/0	5/0

* *Number of plants inoculated over the number that became infected.*

TABLE IV

THE SAP DILUTION EXTINCTION POINTS OF STREAK X AND POTATO X

Virus	Dilution of infected *D. Stramonium* sap				
	10^2	10^3	10^4	10^5	10^6
Streak X	15*/15	15/15	15/15	15/8	15/1
Potato X	10/10	10/10	10/10	10/2	10/0

* *Number of plants inoculated over the number that became infected.*

Thung (6), Salaman (4), Kunkle (2) and others have shown that plants cannot be inoculated with a virus that induces marked symptoms if already infected with one that induces little change from normality if both belong to the same group. Although potato X from local Irish Cobbler induces little change in tomato plants, the presence of this virus completely immunized the tomato plants against streak X. Ten days after inoculating plants with potato X they were re-inoculated with streak X. No symptoms subsequently developed beyond the mild mottle characteristic of potato X. Furthermore, when apparently healthy Irish Cobbler potatoes were inoculated with streak X, ten days later only the ordinary potato X could be recovered by transferring the sap to *D. Stramonium* and tomatoes. On the other hand, when X-free potato seedlings were inoculated with streak X, ten days later transfers of the seedling sap induced the characteristic streak disease on tomatoes and the characteristic mottle and necrosis on *D. Stramonium*.

The precipitin reaction proved also that the virus of streak X belongs to the potato virus X group (3).

Discussion

The discovery of an important streak disease of tomatoes caused by a virus of the potato X group, unassociated with tobacco virus serves to emphasize the economic importance of distinguishing between the virus types or strains

within a group as established by serological and protective inoculation means. It would appear from this study that the ordinary form of potato X is not pathogenic in tomatoes except when associated with "tobacco virus 1", but that forms of the X virus exist that can cause a serious disease of tomatoes. Salaman and his associates (5) have demonstrated that the X virus in potatoes may be a mixture of types. They were able to break up the original X as isolated from potatoes into distinct forms. It would be interesting if a form exists in potatoes that is capable of inducing conspicuous necrosis in tomato. This study indicates that X-free potato seedlings in conjunction with potato plants of any American variety are of value in demonstrating whether a virus of the potato X group is present in a plant other than potatoes. Any form of X may be recovered unchanged from potato seedlings, but from commercial potatoes only the form of X already present can be recovered.

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A STRAIN OF THE VIRUS WHICH CAUSES STREAK IN TOMATO[1]

BY G. H. BERKELEY[2]

Abstract

In this paper the symptoms of a strain of tomato streak virus 1 found in Ontario are described and are compared with those produced by tomato streak virus 1 and tobacco virus 1 on the same hosts. On tobacco, variety White Burley, Adcock, etc., the Ontario strain produces necrotic local lesions on rubbed leaves followed by systemic mottling, whereas tomato streak virus 1 generally produces necrotic local lesions only, though sometimes systemic necrosis may follow. Also, on *Nicotiana sylvestris* the Ontario strain gives rise to systemic mottling with necrosis following the primary necrotic local lesions, whereas tomato streak virus 1 produces primary necrotic local lesions with or without systemic necrosis. The fact that all varieties of tobacco do not react in a similar manner to either tomato streak virus 1 or the Ontario strain is shown by a comparison of symptoms on Harrow Velvet (systemic mottling only), and White Burley or Kelley's (necrotic local lesions sometimes followed by systemic necrosis). On the other hand, all varieties tested responded to inoculation with tobacco virus 1 (tomato mosaic) by production of systemic mottling with some distortion. A series of inoculations on seven varieties of tobacco grown in the field has shown that *N. tabacum*, varieties Adcock, Gold Tip, White Burley and Greenwood, were killed within two weeks after inoculation with tomato streak virus 1, whereas the Ontario strain on the same varieties caused stunting with systemic mottling of leaf tissue, but did not kill the plants. Tests as to reaction to aging and heat have demonstrated that tomato streak virus 1, the Ontario strain of this virus, and tobacco virus 1, have similar properties in that each is viable after six months aging and each is rendered inactive after ten minutes at 90° C. Immunity tests show that tobacco virus 1 immunizes plants against infection with either tomato streak virus 1 or the Ontario strain of this virus. It is suggested, therefore, that tomato streak virus 1 and the Ontario strain of the virus may be strains of tobacco virus 1.

Introduction

Ainsworth, Berkeley and Caldwell (1) have compared tomato viruses as they occur in England and in Canada, and found that tomato streak in both countries was for the most part caused by a single virus which they called tomato streak virus 1, though mixed virus streak also occurred. In most cases where streak was caused by a mixture of viruses they found that one of the viruses concerned was tomato streak virus 1, while the other was a potato virus of the X type. A single case of stem-necrosis streak (Johnson's tobacco virus no. 9) was also encountered in the Canadian material.

Recently Smith (7) has pointed out that tomato streak virus 1 in nature may not always be a pure strain but may consist of two or more closely similar strains. In this respect tomato streak virus 1 is similar to wheat mosaic (4), tobacco mosaic (2), cucumber mosaic (5) and potato X (6, 8) viruses, each of which has been demonstrated to be made up of closely related strains.

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The purpose of the present paper is to describe the symptoms produced by a strain of tomato streak virus 1 found in Ontario, and to compare them with the symptoms produced by tobacco virus 1 and tomato streak virus 1 on the same hosts.

Material and Methods

In June, 1934, a collection of leaves from tomato plants affected with either mosaic or streak was made, and the several viruses obtained therefrom were transferred to *Nicotiana tabacum* (tobacco, 19 varieties), *Lycopersicon esculentum* (tomato, Grand Rapids), *N. glutinosa*, *N. glauca*, petunia, *Fagopyrum esculentum* (buckwheat), *Zinnia elegans* (zinnia), *N. Langsdorfii*, *N. sylvestris* and *N. paniculata*.

Tomato streak virus 1, which was described by Ainsworth, Berkeley and Caldwell (1) as being the cause of single virus streak in both England and Canada, was obtained in Ontario from tomatoes growing under glass and from tobacco growing in the field. A sample of this virus was also received from England through the kindness of G. C. Ainsworth of the Cheshunt Experiment Station. A strain of this virus was also obtained in Ontario from greenhouse tomato and field tobacco. Tobacco virus 1 was obtained from greenhouse and field tomatoes and from field tobacco.

Experimental Results

Symptoms

The results of a large number of symptomatological studies extending over a two-year period are given in Table 1.

It is of particular interest and value to note that tomato streak virus 1 does not produce the same symptom picture on all varieties of *N. tabacum*. On some varieties the symptomatology comprises primary local necrotic lesions with or without secondary systemic necrosis and stunting, whereas in other varieties the primary symptom takes the form of yellow areas followed in a few days by secondary systemic mottling with no necrosis. In the light of these results the necessity of recording the variety of *N. tabacum* used in all experimental tests is obvious.

Tomato streak virus 1 (Ontario strain) produces symptoms identical to those of tomato streak virus 1 on *N. tabacum* varieties Standup Resistant, Halley's and Harrow Velvet, but on *N. tabacum* varieties White Burley, Kelley's, Little Orinoco and Greenwood the primary local necrotic lesions are followed by systemic mottling with distortion instead of systemic necrosis. On *N. sylvestris*, tomato streak virus 1 (Ontario strain) produces local necrotic lesions followed by systemic mottling, the mottled leaves in turn becoming necrotic, whereas tomato streak virus 1 gives rise to local necrotic lesions followed sometimes by systemic necrosis.

Recently Smith (7) has described a green and a yellow strain of tomato streak that differ from the original tomato streak virus 1 and from the strain described in this paper. The green strain as a rule produces on *N. tabacum* (White Burley) no local lesions on inoculated leaves, but when it does, the

TABLE I

COMPARISON OF SYMPTOMS PRODUCED BY TOMATO STREAK VIRUS 1, TOMATO STREAK VIRUS 1 (ONTARIO STRAIN), AND TOBACCO VIRUS 1

Host	Viruses		
	Tomato streak virus 1	Tomato streak virus 1 (Ont. strain)	Tobacco virus 1
N. tabacum, varieties:—			
Standup Resistant, Halley's, Harrow Velvet	Primary yellow lesions + systemic mottling and distortion.	Primary yellow lesions + systemic mottling and distortion.	Primary yellow lesions + systemic mottling and distortion.
White Burley, Kelley's, Little Orinoco, Greenwood, Adcock	Necrotic local lesions ± systemic necrosis.	Necrotic local lesions + systemic mottling and distortion.	Primary yellow lesions + systemic mottling and distortion.
N. glutinosa	Necrotic local lesions.	Necrotic local lesions.	Necrotic local lesions.
N. Langsdorfii	Necrotic local lesions ± systemic necrosis.	Necrotic local lesions ± systemic necrosis.	Necrotic local lesions ± systemic necrosis.
N. sylvestris	Necrotic local lesions ± systemic necrosis.	Necrotic local lesions + systemic mottling with slight necrosis.	Primary yellow lesions + systemic mottling and distortion.
N. paniculata	Primary yellow lesions + systemic mottling and distortion.	Primary yellow lesions + systemic mottling and distortion.	Primary yellow lesions + systemic mottling and distortion.
N. glauca	Distinct systemic mottling of circular spot type.	Distinct systemic mottling of circular spot type.	Indistinct systemic mottling on lower leaves only.
Zinnia elegans	Primary yellow lesions.	Primary yellow lesions.	Primary yellow lesions ± systemic mottling.
Fagopyrum esculentum	Necrotic local lesions (white).	Necrotic local lesions (white).	Systemic mottling.
Petunia	Necrotic local lesions ± systemic necrosis.	Necrotic local lesions ± systemic necrosis.	Systemic mottling.
Tomato (Grand Rapids)	Necrotic lesions on stem, leaves and fruit; mottle and stunting of plant. Under certain conditions the disease may be manifest as a mottle only.	Same as for tomato streak virus 1.	Dark green to light green mottle with leaf distortion but no necrosis.

lesions are followed by severe necrosis. The yellow variant of this strain produces yellow spots on the rubbed leaves. On the other hand the Ontario strain always produces necrotic local lesions on rubbed leaves, followed by systemic mottling. On petunia the Ontario strain produces necrotic local lesions sometimes followed by systemic necrosis, whereas the strains reported by Smith give rise to systemic mottling only.

Whereas the original tomato streak virus 1 and the Ontario strain of this virus described in this paper produced necrotic local lesions on certain varieties of *N. tabacum* (varieties White Burley, Kelley's, Little Orinoco, Greenwood, and Adcock), petunia and *N. sylvestris*, tobacco virus 1 produced no necrotic local lesions on any variety of *N. tabacum*, petunia or *N. sylvestris*, but instead produced systemic mottling with some distortion.

From the above description it is apparent that the three viruses can be readily separated from each other by their reactions on *N. sylvestris*, petunia, and certain varieties of *N. tabacum*.

Table II summarizes the more important differences between tomato streak virus 1, tomato streak virus 1 (Ontario strain) and tobacco virus 1.

TABLE II

DIFFERENTIATION OF THE THREE TOMATO VIRUSES

	Virus of		
	Single virus streak	Single virus streak (Ontario strain)	Tomato mosaic (Common tobacco mosaic)
Local necrotic lesions ± systemic necrosis on *N. tabacum*, varieties White Burley, L. Orinoco, Greenwood, Kelley's and Adcock; and *N. sylvestris;* and petunia.	+	−	−
Local necrotic lesions + systemic mottling on *N. tabacum*, varieties White Burley, L. Orinoco, Greenwood, Kelley's and Adcock; and *N. sylvestris* same but with necrosis following systemic mottling; on petunia local necrotic lesions ± systemic necrosis, but no mottling.	−	+	−
Dark green to light green mottle ± distortion on *N. tabacum*, varieties White Burley, L. Orinoco, Greenwood, Kelley's and Adcock; and *N. sylvestris;* and petunia.	−	−	+

Inoculations under Field Conditions

During the summers of 1935 and 1936 a series of inoculations was made on seven varieties of *N. tabacum* growing outdoors, with the original tomato streak virus 1, tomato streak virus 1 (Ontario strain), and tobacco virus 1. The inoculations were made two weeks after the plants had been transplanted into the field, when they were about eight inches high. Very striking results were obtained in that several varieties were killed by tomato streak virus 1, whereas tomato streak virus 1 (Ontario strain) produced local necrotic lesions followed by systemic mottling and stunting of plants. Tobacco virus 1 produced only systemic mottling and stunting on all varieties.

TABLE III

DESCRIPTION OF SYMPTOMS ON *N. tabacum* OUTDOORS

Host, *N. tabacum* variety	Type	Tomato streak virus 1	Tomato streak virus 1 (Ontario strain)	Tobacco mosaic virus 1
Adcock Gold Tip W. Burley Greenwood	FC* FC B DTT	Necrotic local lesions + systemic necrosis followed by death of plant within 20 days.	Necrotic local lesions + systemic mottling. Plants not killed.	Primary yellow lesions + systemic mottling.
H. Velvet Halley's Standup Resistant	B B B	Primary yellow lesions + systemic mottling.	As in tomato streak virus 1.	As in tomato streak virus 1.

* FC = *Flue cured;* B = *Burley;* DTT = *Dark tobacco type.*

The fact that tomato streak virus 1 killed several varieties of tobacco within 20 days after they had been inoculated, while the Ontario strain of this virus produced on the same varieties only necrotic local lesions and systemic mottling with stunting, points out a still further difference between these two viruses, (Plate III, Figs. 1, 2 and 3).

Properties of the Viruses

It is interesting to note that tomato streak virus 1 and the Ontario strain of this virus have properties similar to tobacco virus 1. This would indicate that tomato streak virus 1 is closely related to tobacco virus 1, even though certain host reactions may be dissimilar. See Table IV.

TABLE IV

PROPERTIES OF THE VIRUSES

	Aging: viable after 6 months	Resistance to heat: Not viable after 10 min. at 90° C. but viable at 80° C.
Tomato streak virus 1	+	+
Tomato streak virus 1 (Ontario strain)	+	+
Tobacco virus 1	+	+

Immunity Tests

It is now generally accepted that systemic infection of a plant with a virus precludes the entrance into that plant of another strain of the same, or a very closely related virus. Therefore, if immunity of this type can be demonstrated it follows that the viruses concerned are very closely related, if not strains of the same virus. Hence, tests were made to ascertain whether or not tobacco virus 1 would establish immunity in a plant against a later inoculation with either tomato streak virus 1 or the Ontario strain of this virus.

These tests demonstrated that when plants of *N. tabacum*, variety Adcock, were inoculated with tobacco virus 1 alone, systemic infection resulted, and when these systemically infected plants were later inoculated with either tomato streak virus 1 or the Ontario strain of this virus, no further symptoms developed, indicating that immunity had been established, since the check plants (inoculated with streak virus alone) produced necrotic local lesions on the rubbed leaves, as was to be expected.

These results indicate a very close relationship between the three viruses under discussion.

Discussion

Evidence is given which demonstrates that all varieties of tobacco do not necessarily respond in a similar manner to a given virus. In so far as tobacco virus 1 is concerned, all varieties have responded in a like manner, that is, by the production of primary yellow lesions followed by systemic mottling. However, although tomato streak virus 1 produces similar symptoms in some varieties of *N. tabacum*, in others, namely Kelley's, Little Orinoco, Greenwood, Adcock and White Burley, it produces local necrotic lesions with or without systemic necrosis, while in the case of tomato streak virus 1 (Ontario strain), the same varieties react by producing local necrotic lesions followed by systemic mottling.

It would appear that the Ontario strain of tomato streak virus 1 is intermediate in its reactions between tomato streak virus 1 on the one hand, and tobacco virus 1 on the other, since on *N. tabacum* (variety Greenwood, Kelley's, Adcock, White Burley and Little Orinoco) and *N. sylvestris* necrotic local lesions are followed by systemic mottling, whereas no such systemic mottling is general with tomato streak virus 1, and systemic mottling only occurs with tobacco virus 1.

The fact that these two streak viruses have properties (aging and resistance to heat) similar to those of tobacco virus 1, and the further fact that tobacco virus 1 is capable of immunizing plants against either of these streak viruses would indicate that tomato streak virus 1, and the Ontario strain of this virus may be strains of tobacco virus 1.

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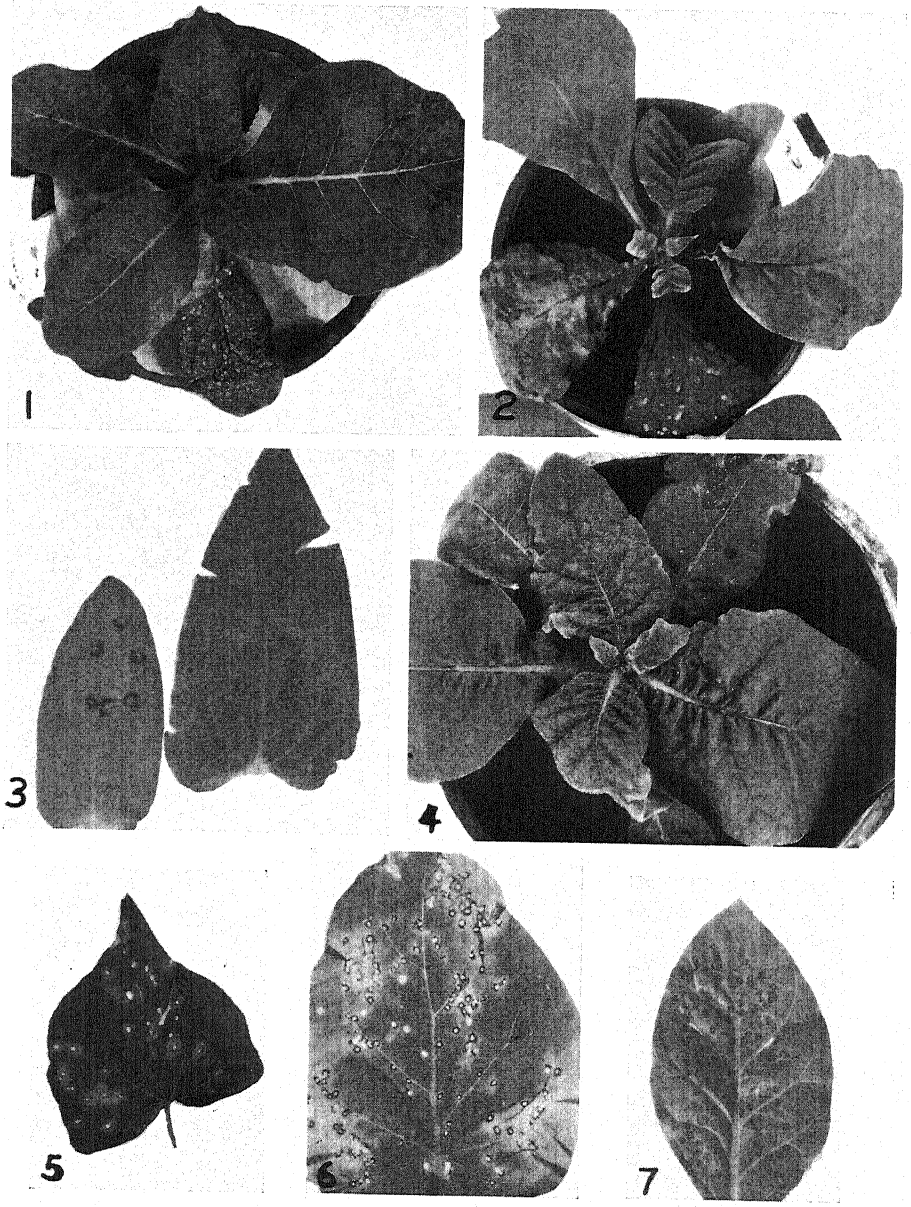
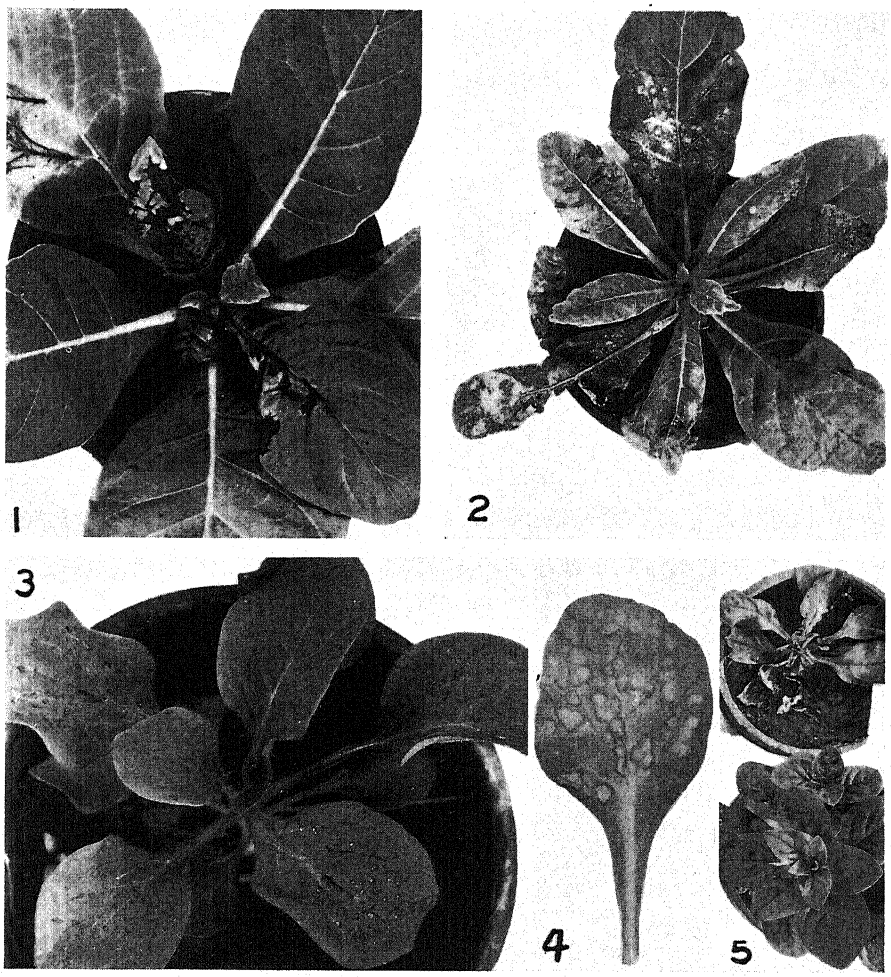
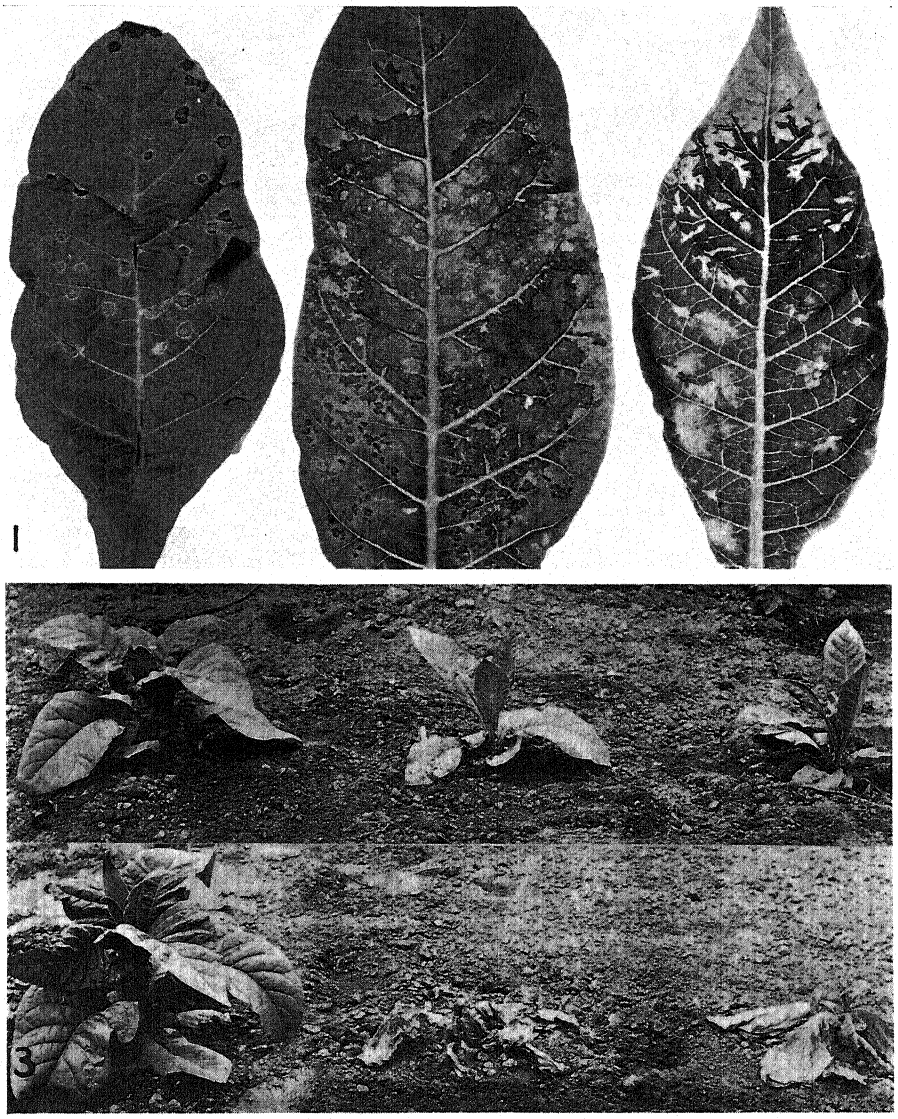


Tomato streak virus 1:—FIG. 1, *showing necrotic local lesions and systemic mottling as produced by Ontario strain on N. tabacum, var. White Burley.* FIG. 2, *showing necrotic local lesions and systemic necrosis as produced by tomato streak virus 1 on N. tabacum var. White Stem Orinoco.* FIG. 3, *yellow local lesions produced on zinnia by either tomato streak virus 1 or the Ontario strain. On a few occasions a slight necrosis developed in the yellow lesion.* FIG. 4, *showing necrotic local lesions and systemic necrosis (slight) on N. Langsdorfii.* FIG. 5, *white necrotic local lesion produced on buckwheat.* FIG. 6, *necrotic local lesions on N. glutinosa.* FIG. 7, *necrotic local lesions on N. tabacum, var. White Burley.*



Tomato streak virus 1:—FIG. 1, *showing necrotic local lesions and systemic necrosis produced by tomato streak virus I on N. sylvestris.* FIG. 2, *showing necrotic local lesions and systemic mottling with necrosis as produced on N. sylvestris by Ontario strain of tomato streak virus 1.* FIG. 3, *necrotic local lesions as produced on petunia by either the tomato streak virus 1 or the Ontario strain.* FIG. 4, *large local lesions on petunia sometimes produced when mature lower leaves are inoculated.* FIG 5, *upper, showing systemic necrosis on petunia as produced by either tomato streak virus 1 or the Ontario strain; lower, systemic mottling as produced by tobacco virus 1.*



Tomato streak virus 1:—All figures of tobacco, variety Adcock, growing in the field. FIG. 1, *left, shows necrotic local lesions on inoculated leaf, centre, systemic mottling with necrosis on same plant; right, systemic mottling only on tip leaves of same plant. All three symptoms the result of inoculation with Ontario strain of tomato virus 1.* FIG. 2, *tomato streak virus 1 (Ontario strain); left, healthy check, and two plants to right show necrotic local lesions with systemic mottling. Note dwarfing.* FIG. 3, *tomato streak virus 1; left, healthy check; centre and right, two inoculated plants. Note inoculated plants are dead. First symptoms on these plants comprised necrotic local lesions, followed later by systemic necrosis.*

VARIETAL TESTING FOR THE REACTION OF OATS TO DISEASES, ESPECIALLY COVERED SMUT[1]

By O. S. Aamodt[2] and A. W. Platt[3]

Abstract

Artificial inoculation of oat varieties was carried on for a period of five years. Sixty-one varieties of oats were tested in replicated plots for two years and thirteen for three years with a composite collection of inoculum of the covered smut organism, *Ustilago levis* (Kell. and Swingle) Mag. All gradations in reaction from high susceptibility to apparent immunity were found to exist.

Dehulling the kernels previous to inoculation increased the incidence of smut approximately six times. Susceptible varieties gave relatively greater increases in smut when dehulled than did resistant varieties. The increase in smut obtained by dehulling was independent of the year in which the test was conducted.

There were no significant differences in the total amount of smut obtained between any two of the seasons in which the tests were conducted but the season influenced the relative varietal reaction.

Natural epidemics of halo-blight *(Pseudomonas corona-faciens* (Ch. Elliott) Stev.) and blast (cause unknown) provided opportunities for obtaining data on the reaction of a large number of oat varieties. Immunity from neither of these diseases was observed but marked varietal differences were noted.

Introduction

As a prerequisite to a breeding program for disease resistance in oats, it was desirable to obtain information on the reaction of varieties to the diseases present in Alberta. According to the reports on the "Prevalence of Plant Diseases in Canada" (3, 4, 15) the most common and destructive diseases of oats in Alberta are probably covered smut, caused by *Ustilago levis* (Kell and Swingle) Mag., halo-blight, caused by *Pseudomonas coronafaciens* (Ch. Elliott) Stev., and blast, the specific cause of which is unknown. It is reported that from 20 to 46% of the fields examined for covered smut were found to contain smut and that percentages as high as 30 were recorded. Halo-blight is reported as common practically every year, with the severity of the attack and the consequent damage varying with the year from no apparent damage to light general damage. Not much information is available as to the loss in yield attributable to this disease.

Covered Smut *(Ustilago levis)*

Literature Review

No attempt has been made to review in any detail the literature on covered smut of oats. That portion of the literature dealing with aspects of the subject under discussion in this paper will be mentioned.

[1] *Manuscript received September 26, 1936.*

Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada. This paper was presented, in part, before the Canadian Phytopathological Society Meeting at Edmonton, June, 1935.

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Reed (16) in 1920 reviewed the work of earlier authors on the subject of the comparative resistance of oat varieties to covered smut. In his own work he tested the reaction of seven host species and found that *Avena brevis*, Roth. and *A. strigosa* Schib. had smut-free plants, while *A. byzantina* C. Koch. was highly resistant. All other species were generally susceptible, although two varieties of common black oats were apparently immune Gaines (10) tested the reaction of some 210 varieties to covered smut and found that 21 of these were smut-free.

Dehulling the seed before inoculation has been shown by several workers (2, 11, 14, 19) to increase the amount of smut obtained. Johnston (14) found that this increase was greatest in susceptible varieties and least in resistant varieties.

Methods

Preliminary tests on the reaction of oat varieties to covered smut were commenced at Edmonton in 1930. In that year hulled* kernels of several oat varieties were inoculated with a composite collection of chlamydospores of the covered smut fungus, and seeded in 10-foot rows, 100 seeds per row. In 1931 the varieties were tested, both with the hull of the kernel removed before inoculation and with the hull of the kernel intact. These treatments were not replicated. In 1932, and in subsequent years, the treatments were similar to those described for 1931, except that they were in duplicate. In all tests the varieties were planted in a systematic order. This is not considered by the writers to invalidate the analysis of the data by variance, for differences due to positional effect are not likely to influence smut infection.

The inoculum used was a composite collection of smutted panicles gathered from places throughout the province and from the varietal tests at this station. The smutted panicles were of the covered type. The chlamydospores when examined under the microscope appeared to be typical *U. levis*. The smutted panicles gathered were passed through a meat grinder and the coarser chaff removed by sifting through a screen. Liberal and approximately equal amounts of inoculum were added to each lot of seed.

Reed (17) has shown that physiologic forms of *U. levis* exist. A detailed study of distribution and prevalence of physiologic forms of *U. levis* has not been made in Alberta. It is believed that the composite inoculum used in this investigation was a representative sample of the forms prevalent in the province.

The senior author (1) has discussed the use of composite cultures in testing the reaction of wheat varieties to *Tilletia levis* and *T. tritici*. In that investigation the results indicated that the use of composite collections of chlamydospores of the bunt fungi in testing the reaction of wheat varieties gave results that were sufficiently reliable for all practical purposes. While

* *Throughout this paper the current practice is being followed of referring to normal grain with the hull intact as "hulled", and to grain from which the hull has been removed artificially as "dehulled".*

the use of composite inoculum may be expected to bring in a possible deviation in the smut percentage from that which might have been obtained had pure cultures been used, it is believed that their use is warranted in establishing the relative susceptibility or resistance of oat varieties to covered smut.

The effect of variations in spore load were not studied in this investigation. Gaines (9) cites the work of Heald who showed that up to a certain maximum load, infection in wheat increases with an increase in spore load. In this investigation the aim has been to have the spore load above the optimum, and in this way eliminate a portion of the variations due to this factor. However, further investigations on the effect of spore load on covered smut infection in oats would appear to be well worth while.

Seeding was done each spring when the soil temperatures reached 60–65° F., as these temperatures have been shown by Johnston (14) to be the most favorable for infection.

At harvest time the plants were pulled and classified as smutted or smut-free. The percentage of smutted plants was calculated for each row.

Results

As the varieties were not replicated in the preliminary experiments of 1930 and 1931, the data obtained on varietal reaction in these tests will not be presented in this paper. These data showed, however, that there were marked varietal differences in reaction to covered smut. The varieties of *A. nuda* that were tested showed high susceptibility, the percentage of plants smutted ranging from 64 to 100. Those of *A sativa orientalis* were generally susceptible but less so than those of *A. nuda*. The varieties of *A. byzantina*, on the other hand, appeared quite resistant while those of *A. sativa* ranged from high susceptibility to apparent immunity.

Some 61 varieties representing various species were selected for further study. All of these varieties were tested for smut reaction in both 1932 and 1933, while 13 that were more promising from an agronomic standpoint were tested again in 1934.

Data on the smut reaction of 11 varieties of *A. sativa* and 2 varieties of *A. sativa orientalis* for the three-year period, 1932–34, are presented in Table I, while data on the smut reaction of 41 varieties of *A. sativa* and 7 varieties of *A. byzantina* for the two-year period, 1932–33, are presented in Table II. In Table I the mean smut percentage for the two-year period 1932–33 is given in addition to the mean smut percentage for the three-year period, 1932–34. Thus the smut percentage of any of the 13 varieties listed in this table is directly comparable with those of any of the 48 varieties listed in Table II. In view of the different infection rates of hulled and dehulled seed, which are discussed later in the paper, the standard errors of the individual treatment averages, as well as the general mean for each variety, are given below Tables I and II. This will enable the reader to estimate readily the significance of the observed differences between varieties obtained with either hulled or dehulled seed.

TABLE I

COVERED SMUT, HALO-BLIGHT AND BLAST REACTIONS OF OAT VARIETIES

Variety	C.A.N.†	Percentage covered smut*											% halo-blight			% blast
		Hulls not removed					Hulls removed									
		1932	1933	1934	Av. 1932–33	Av. 1932–34	1932	1933	1934	Av. 1932–33	Av. 1932–34	Mean 1932–33	Mean 1932–34	1930	1931	Av.	1929
Avena sativa																	
Victory	518	12.5	34.0	12.5	23.3	19.7	69.5	79.5	74.5	74.5	74.5	48.9	47.1	25	25	25.0	12.3
Banner	62	5.5	28.5	15.5	17.0	16.5	55.0	79.0	65.0	67.0	66.3	42.0	41.4	5	8	6.5	—
Legacy	460	4.0	15.5	10.5	9.8	10.0	73.5	66.5	68.0	70.0	69.3	39.9	39.7	—	—	—	—
Alaska	458	10.5	6.0	16.5	8.3	11.0	67.5	59.5	61.0	63.5	62.7	35.9	36.8	15	15	15.0	—
White Cross	601	1.5	7.5	15.5	4.5	8.2	61.5	28.5	71.0	45.0	53.7	24.8	30.9	20	12	16.0	24.0
Gopher	14	2.0	3.0	7.5	2.5	4.2	46.0	22.5	67.0	34.3	45.2	18.4	24.7	5	15	10.0	24.0
Accession No. 1	—	11.5	0.5	2.0	6.0	4.7	29.0	41.5	39.5	35.3	36.7	20.6	20.7	—	—	—	—
Accession No. 2	—	14.0	0.0	0.0	7.0	4.7	12.0	5.5	6.5	8.8	8.0	7.9	6.3	—	—	—	—
Accession No. 3	—	1.0	0.0	0.0	0.5	0.3	3.0	4.5	1.0	3.8	2.8	2.1	1.6	—	—	—	—
O.A.C. 144	39	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10	10	10.0	—
Markton	67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10	12	11.0	11.9
A. sativa orientalis																	
New Era	181	13.0	13.0	29.5	13.0	18.5	90.5	85.5	93.5	88.0	89.8	50.5	54.2	—	—	—	—
Leader	199	23.0	25.5	23.5	24.3	24.0	74.0	72.5	80.5	73.3	75.7	48.8	49.8	15	18	16.5	—

	Hulls not removed	*Hulls removed*	*General mean*
S.E. of percent covered smut infection, 1932–34, is	*±2.31*	*±4.89*	*±2.71*
S.E. of the difference of two such means is	*±3.27*	*±6.81*	*±3.83*

† *Canadian Accession number.*
* *Each value is the average of duplicate determinations.*

TABLE II

COVERED SMUT, HALO-BLIGHT AND BLAST REACTIONS OF OAT VARIETIES

Variety	C.A.N.*	Percentage covered smut**							% halo-blight			% blast, 1929
		Hulls not removed			Hulls removed			Mean 1932–33				
		1932	1933	Av.	1932	1933	Av.		1930	1931	Av.	
A. sativa												
Accession No. 4	—	22.5	0.5	11.5	74.5	89.0	81.8	46.6	—	—	—	—
Rainbow	361	1.5	27.0	14.3	76.5	79.0	77.8	46.0	—	—	—	—
Colorado No. 37	301	0.0	21.5	10.8	74.5	72.5	73.5	42.1	25	12	18.5	25.6
Russian No. 76	448	9.0	13.0	11.0	81.0	65.0	73.0	42.0	—	—	—	—
Star	467	12.0	12.0	12.0	53.5	57.0	55.3	33.6	—	—	—	—
Cole	493	5.5	10.0	7.8	54.0	51.5	52.8	30.3	5	5	5.0	10.9
Iogold	139	0.0	9.5	4.8	60.0	25.0	42.5	23.6	8	3	5.5	10.3
Richland	4	0.0	9.5	4.8	5.0	65.0	35.0	19.9	20	20	20.0	21.0
Iowar	5	0.0	3.0	1.5	35.0	39.5	37.3	19.4	3	5	4.0	11.6
Madrid	325	2.0	1.5	1.8	36.5	34.0	35.3	18.5	3	8	5.5	31.3
Iowar	—	4.0	3.0	3.5	15.0	39.5	27.3	15.4	—	—	—	—
Kherson	255	0.0	2.0	1.0	20.5	28.0	24.3	12.6	—	—	—	—
Green Russian	246	3.0	1.5	2.3	25.0	16.5	20.8	11.5	5	10	7.5	18.0
North Finnish	510	3.0	0.0	1.5	20.0	17.0	18.5	10.0	5	10	7.5	5.5
Terry	282	1.5	0.0	0.8	17.0	21.0	19.0	9.9	5	8	6.5	9.2
Loviscaptonia	—	2.5	0.0	1.3	9.0	10.0	9.5	5.4	—	—	—	—
Accession No. 1	—	2.0	1.5	1.8	11.0	7.0	9.0	5.4	—	—	—	—
White Maine	289	0.5	1.5	1.0	7.5	4.0	5.8	3.4	10	10	10.0	21.0
Keystone	254	0.0	0.0	0.0	9.0	1.5	5.3	2.6	3	5	4.0	7.5
Svalof	—	1.0	0.0	0.5	5.5	0.0	2.8	1.6	5	8	6.5	—
Ferguson Navarro	234	0.0	0.0	0.0	6.0	0.0	3.0	1.5	0	5	2.5	34.5
Scottish Chief	275	0.0	0.0	0.0	2.0	2.5	2.3	1.1	25	12	18.5	17.7
Dwarf Culberson	231	0.5	0.0	0.3	4.0	0.0	2.0	1.1	0	3	1.5	4.8
Hatchett	247	1.0	0.5	0.8	2.0	0.0	1.0	0.9	3	3	3.0	3.7
Black Bell No. 1	176	0.0	0.0	0.0	1.5	2.0	1.8	0.9	0	8	4.0	16.3
Monarch	260	0.0	0.0	0.0	3.0	0.0	1.5	0.8	10	10	10.0	6.9
Yellow Russian	476	0.0	0.0	0.0	2.0	1.0	1.5	0.8	3	8	5.5	—
Pringles Progress	267	0.5	0.0	0.3	2.0	0.0	1.0	0.6	50	50	50.0	12.9
O.A.C. No. 72	468	0.0	0.0	0.0	1.5	0.0	0.8	0.4	3	3	3.0	—
Bicknell	223	0.0	0.0	0.0	1.0	0.0	0.5	0.3	3	3	3.0	6.5
Kanota	253	0.0	0.0	0.0	1.0	0.0	0.5	0.3	3	3	3.0	2.0
Aurora	64	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	3	1.5	24.9
Awnless Monarch	60	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25	15	20.0	7.9
Black Diamond	115	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15	12	13.5	9.8
Black Mesdag	432	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10	20	15.0	2.6
Cornellian	435	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10	10	10.0	26.5
Cornellian	302	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	8	5.5	15.4
Early Ripe	496	0.0	0.0	0.0	0.0	0.0	0.0	0.0	25	20	22.5	—
Frazier	233	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3	3	3.0	1.2
Nortex	263	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	3	1.5	5.8
Teck	280	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	3	1.5	—
A. byzantina												
Awnless rustproof	160	0.0	0.0	0.0	21.0	2.5	11.8	5.9	25	20	22.5	0.3
Fulghum	2	0.0	0.0	0.0	0.5	1.0	0.8	0.4	5	8	6.5	0.7
Black Algerian	174	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	1	0.5	4.4
Burt	299	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5	5	5.0	8.4
Cassel	452	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	8	4.0	—
Red Algerian	270	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10	8	9.0	2.1
Red rustproof	514	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	1	0.5	5.6

	Hulls not removed	*Hulls removed*	*General mean*
**Canadian Accession Number.*			
***Each value is the average of duplicate counts.*			
S.E. of the mean percent covered smut is	*±1.80*	*±3.66*	*±2.03*
S.E. of the difference of two such means is	*±2.54*	*±5.18*	*±2.87*

The analysis of variance as developed by Fisher (8) has been used for the treatment of the data obtained in this investigation. An analysis of variance was calculated from the data obtained from the 61 varieties listed in Tables I and II for the two-year period 1932–33. The results are presented in Table III. In addition, an analysis of variance was calculated from the data obtained from the 13 varieties listed in Table I for the three-year period, 1932–34. The results are presented in Table IV. The significance of the mean square of any possible variant compared with the mean square for error, was determined by the method given by Snedecor (18). As the results obtained in each of the analyses are quite similar, they will be discussed together as far as possible.

The magnitude of the mean squares for varieties in relation to that of the mean squares for error (Tables III and IV) indicates that all varieties are not genetically alike in their reaction to covered smut. When comparing the mean smut percentage for the period 1932–34 of the varieties listed in Table I, it will be noted that Victory, Banner and Legacy were highly susceptible; White Cross and Gopher were significantly lower in mean smut percentage than the above three varieties, but were nevertheless quite susceptible. Alaska was significantly more susceptible than Gopher but less so than Victory. O.A.C. 144 and Markton appeared to be immune from the covered smut collections used in these investigations. The two varieties of *A. sativa orientalis*, New Era and Leader, were both highly susceptible. When considering the mean percentage smut for the two-year period 1932–33 of the varieties listed in Tables I and II, it will be noted that the varieties of *A. sativa* range from high susceptibility to apparent immunity. Of the 52 varieties representing this species, six had a mean smut percentage of over 40%, seven had 20 to 40%, seven had 10 to 20%, twelve had 1 to 10%, eight had less than 1% and twelve failed to show any smut. Five of the seven varieties of *A. byzantina* tested were apparently immune, while the remaining two were highly resistant.

The results obtained show that there is already in existence a wealth of varieties that possess a high degree of resistance to covered smut. Unfortunately none of these varieties are agronomically suitable for Alberta. The most commonly grown variety, Victory, was one of the most susceptible varieties tested, while Gopher, which is the least susceptible of the recommended varieties, was moderately susceptible, having 24.7% infection. With these results in mind it is not difficult to understand why it is that covered smut is so prevalent in commercial oat fields.

The smut obtained on plants grown from hulled seed was much less than that obtained on plants grown from dehulled seed. That this difference in smutted plants is statistically significant is shown by the highly significant mean squares obtained for treatments compared with those obtained for errors (Tables III and IV). The mean smut percentages obtained on 61 varieties for the two-year period 1932–33 were 3.5%, for hulled seed, and 21.3% for dehulled seed. The thirteen varieties grown for the three-year

TABLE III

ANALYSIS OF VARIANCE OF PERCENTAGE COVERED SMUT ON OAT VARIETIES, 1932–33

Variation due to	Degrees of freedom	Sums of squares	Mean square	F
Years	1	11	11	0.2
Treatments	1	38,669	38,669	1171.7*
Varieties	60	131,620	21,940	664.8*
Varieties × years	60	3,412	57	1.7*
Varieties × treatments	60	63,503	1,058	32.1*
Treatments × years	1	126	126	3.8
Varieties × treatments × years	60	9,126	152	4.6*
Error	244	8,111	33	–
Total	487	254,578		

* *Exceeds the 1% point.*

TABLE IV

ANALYSIS OF VARIANCE OF PERCENTAGE COVERED SMUT ON OAT VARIETIES, 1932–34

Variation due to	Degrees of freedom	Sums of squares	Mean square	F
Years	2	352	176	2.0
Treatments	1	49,541	49,541	563.0*
Varieties	12	56,633	4,719	53.6*
Varieties × years	24	4,578	191	2.2*
Varieties × treatments	12	23,260	1,938	22.0*
Treatments × years	2	252	126	1.4
Varieties × treatments × years	24	2,418	101	1.1
Error	78	6,838	88	–
Total	155	143,872		

* *Exceeds the 1% point.*

period 1932–34 showed percentages of 9.3 and 45.0 for hulled and dehulled seed respectively. These results are in agreement with those obtained by other workers (2, 11, 14, 19).

The increase in smut that may be expected, due to the removal of the hull, is dependent on the varieties used. This is shown by the significant mean squares obtained for the interaction of varieties and treatments compared with those obtained for errors (Tables III and IV). That is, the varietal differences were not the same under the two conditions of treatment, namely, hulled and dehulled seed. The differences given in Table V between the percentages of smut in the two treatments for each variety were obtained by subtracting the smut percentage, calculated by totalling the means of duplicate determinations for three years, of hulls not removed from that obtained in a similar manner with hulls removed. The standard error of the difference between two such differences would be $\sqrt{\frac{88 \times 3 \times 2 \times 2,}{2}}$ or 22.98. Cross

differences in excess of 45.96 may be judged to be significant. Thus the cross differences between all the more susceptible varieties and Accessions No. 2 and No. 3 are significant.

These results clearly show that the susceptible varieties gave relatively greater increases in smut when dehulled than did the resistant varieties, indicating a more favorable response to dehulling. These results agree with those obtained by Johnston (14) who has shown also that certain oat varieties owe their apparent resistance to the mechanical protection afforded by their hulls, while other varieties have resistance that is independent of the presence or absence of the hull. Such results are substantiated in the present investigation. In all cases where smut occurred, the percentage smut obtained when the hull was removed was greater than that obtained when the hull was intact, except in the case of Accession No. 2 in 1932. Thus it would appear that in these varieties the hull acts as a protective organ. Other varieties, however, failed to show smut regardless of whether the hull had been removed or not. It would therefore appear that this latter group has resistance to, rather than protection from smut infection.

TABLE V

DIFFERENCES BETWEEN TOTAL SMUT PERCENTAGES WITH HULLS REMOVED AND HULLS NOT REMOVED*

Variety	Difference
Victory	164.5
Banner	149.5
Legacy	170.0
Alaska	155.0
White Cross	136.5
Gopher	123.0
Accession No. 1	96.0
Accession No. 2	10.0
Accession No. 3	7.5
O.A.C. 144	0
Markton	0
New Era	214.0
Leader	155.0

** Total differences for three years of the mean of duplicate determinations.*
Cross differences in excess of 45.96 may be judged to be significant.

Johnson (13) has shown that it is possible to transfer the resistance of agronomically inferior varieties to agronomically superior varieties without difficulty. The question arises as to whether the plant breeder should use hulled or dehulled seed, or both, in testing the reaction of varieties and hybrid selections to covered smut. The production of new smut-resistant varieties should have as its objective the production of varieties that have inherent physiological resistance rather than mere mechanical protection from infection, since a variety having only mechanical protection may become infected when grown commercially, owing to injuries sustained by the hull during threshing operations. The writers have not failed to recognize that there may be also a mechanical barrier to natural inoculation in the field when the caryopsis is developing within the hull and the smut spores are being blown about by the wind. The high percentage of smut commonly observed in commercial oat fields compared with that obtained in artificial tests using hulled seed, suggests that threshing injuries sustained by the hull must be one of the chief factors involved. Resistance in a variety or hybrid population

can be determined with surety only when the complicating factor of the mechanical protection of the hull is removed. Dehulling sufficient kernels to obtain reliable indices of infection is both tedious and costly, when a large number of varieties or hybrid selections are to be tested. Such a procedure is to be avoided if possible. An examination of the data in Tables I and II reveals that when tested for two years, 1932–33, only eleven of the 61 varieties failed to show smut when grown from hulled seed, but these smutted when grown from dehulled seed. Consequently, it would seem that when the object of the investigation is the discovery of resistance in a variety or hybrid selection, it would be a good procedure to test all the material, using hulled seed, eliminating any that are smutted and retesting the remainder, using dehulled seed.

There were no significant differences in the total amount of smut obtained between any two of the seasons in which the tests were conducted. This is shown by the non-significant mean squares for years compared with those for errors, (Tables III and IV). It would appear that the environmental factors that influence the incidence of this disease were sufficiently constant over the three-year period of this test not to influence the amount of smut obtained.

The mean squares obtained for the interaction of years and treatments compared with those obtained for errors were not significant (Tables III and IV). This indicates that the increase in smut obtained by removing the hull is independent of the year in which the test was made. If conditions were such that the amount of smut obtained varied markedly with the year, it is possible that this relation would not hold.

When considering the data obtained on the 13 varieties tested for the three-year period 1932–34 it was found that the relative varietal reaction was influenced by the season, as is shown by the significant mean square obtained for interaction (Table IV). The F value for the comparison of the mean square of varieties with that of varieties $\times$ years ($F = 24.7$) greatly exceeds the 1% point. This shows that varietal reaction generally was consistent from year to year for most of the varieties. The data in Table III, for the 61 varieties tested for the two year period 1932–33, show that the mean square of varieties $\times$ years compared with that of error was also significant.

The significant mean square for interaction that was obtained means that certain varieties reacted differently to the conditions obtaining in any given year than did other varieties. By calculating the difference in percentage smut for each variety from the percentage smut of the same variety in the other two years, with a uniform correction being made for the average difference between all varieties in that year and the average of these varieties in the other years, and testing the cross differences obtained, it can be shown which varieties are reacting in a differential manner in each of the years. Such cross differences were calculated for the 13 varieties grown for the three-year period 1932–34. The data obtained are presented in Table VI. The standard error of the difference between any two cross differences is 27.6,

hence cross differences in excess of 55.2 may be considered significant. Accession No. 2 had a plus difference of 46.1 in 1932, while Banner had a minus difference of 60.9. The cross difference of 107.0 is highly significant, and clearly indicates a differential response. Thus conditions in 1932 seemed to be very suitable for infection of Accession No. 2 and unsuitable for Victory. In 1933 conditions were such as to favor infection and smut development on Victory and Banner, and under the same conditions infection was not favored on White Cross and Gopher. On the other hand, in 1934, this latter situation was reversed, as infection of White Cross and Gopher was favored and infection of Victory and Banner was not.

TABLE VI

DEVIATION OF SMUT PERCENTAGE OF FOUR COUNTS OF THE VARIETIES IN A GIVEN YEAR FROM THE AVERAGE SMUT PERCENTAGE OF THE SAME VARIETY IN THE OTHER TWO YEARS, MINUS THE DIFFERENCE BETWEEN THE AVERAGE SMUT PERCENTAGE OF ALL VARIETIES FOR THAT YEAR AND THE AVERAGE SMUT PERCENTAGE OF ALL VARIETIES IN THE OTHER TWO YEARS

Variety	Year		
	1932	1933	1934
New Era	− 8.4	−22.9	+31.3
Leader	− 1.9	− 1.4	+ 3.3
Victory	−30.4	+64.6	−34.2
Banner	−60.9	+80.6	−19.7
Legacy	+ 9.6	+14.6	−15.2
Alaska	+19.1	−17.9	− 1.2
White Cross	+ 9.6	−70.9	+61.3
Gopher	+ 2.1	−64.9	+62.8
Accession No. 1	+ 3.6	+ 8.8	−12.4
Accession No. 2	+46.1	−14.9	−31.2
Accession No. 3	+ 8.6	+10.6	−19.2
O.A.C. 144	+ 6.1	+ 6.6	−12.7
Markton	+ 6.1	+ 6.6	−12.7

The standard error of the difference between one total and the average of the others is 19.52.

The standard error of the difference of two such differences is 27.6.

Cross differences in excess of 55.2 may be judged to be significant.

The second order interaction of varieties × treatments × years for the 61 varieties tested for the two-year period 1932–33 is significant (Table III). This indicates that the differential effect of dehulling on the resistant and susceptible varieties was not consistent for the two years of the test. In Table IV the data show that for the 13 varieties tested for the three-year period 1932–34, the second order interaction was not significant.

The nature of the conditions that bring about these differential responses can only be a subject for speculation at the present time. Possibly the relation between the environmental factors in the maturation of the seed, and the growth rate of the varieties as affected by the season following infection are related factors. Whatever the reason for these differential reactions the fact is apparent that a much better index of the reaction of a given variety may be obtained when that variety is tested over a period of years, rather than in a single season.

Halo-Blight (*Pseudomonas coronafaciens*)

Literature Review

Investigations concerning varietal reaction of oats to halo-blight have been reported by Elliott (5). She was able to demonstrate that varieties differed in their reaction to this disease, but that all the varieties tested were attacked

to some extent and that the differences between varieties tended to become less marked as the severity of the attack was increased or as the season advanced.

Results

In 1930, and again in 1931, a natural epidemic of halo-blight occurred in the oat classification nursery at this station. Notes were taken on the reaction of each of the 188 varieties growing in the nursery by estimating the percentage of leaf area affected. The data obtained on those varieties, upon which a smut reaction was obtained also, are presented in Tables I and II. As the varieties were not replicated it is difficult to judge the significance of the results obtained. However, a highly significant coefficient of correlation of +.797 was secured between the indices of blight obtained in 1930 and those obtained in 1931. This indicates that the varietal reaction was reasonably consistent over this two-year period and that at least the larger differences between varieties, in amount of blight, may be regarded as significant.

In 1930 several of the varieties failed to show any symptoms of the disease, but in 1931 lesions were present on all of the varieties. It is thus apparent that immunity from this disease was not present in any of the varieties that were subjected to the epidemic. These results are similar to those obtained by Elliott (5). Moreover, there did not appear to be any distinct difference between the reactions of the various species tested. Lee, C.A.N. 255, a variety of *A. sativa*, Chinese Hulless, C.A.N. 225, a variety of *A. nuda*, and Black Algerian, C.A.N. 174, Culred, C.A.N. 454, and Red Rustproof, C.A.N. 514, varieties of *A. byzantina* appeared to be the most resistant, being blighted less than 1% on the average. Gartons' Yellow, C.A.N. 240, a variety of *A. sativa orientalis*, appeared to be the most susceptible, being given a rating of 72.5% blighted. Pringle's Progress C.A.N. 267, Upright C.A.N. 517, and Columbian C.A.N. 465, varieties of *A. sativa*, were also quite susceptible, having an infection rating of 50, 50 and 65% respectively.

It is interesting from a taxonomic point of view to note the difference in the amount of blight obtained on the common varieties Victory and Banner. Victory had 25.0% blight, while Banner had only 6.5%. It is difficult to distinguish between these varieties morphologically, but when an epidemic of halo-blight is present Victory is readily distinguishable from Banner by the greater number of halo-blight lesions present on the leaves.

Blast

Literature Review

The literature on blast has recently been reviewed by Huskins (12) and in consequence will not be dealt with at any length in this paper.

Elliott (6), while noting that blast was often associated with halo-blight in the field, showed that blast was not due to the blight but was probably favored by the same environmental factors. As a result of further work (7) she was able to show that varieties differed in the amount of blasting and that, while the amount of blasting varied greatly in different years, the relative

varietal reaction was reasonably consistent. Huskins (12) has called attention to possible genetic resistance to blast in certain oat varieties and to the possibility that these differences are not due to a general physiological correlation with panicle size.

Results

In 1929 a large amount of blasting occurred on the oat varieties grown at this station. Part of the data obtained on varietal reactions* are presented in Tables I and II. While these data are not sufficiently complete to allow for statistical evaluation it would appear that distinct varietal differences exist. There is a difference of approximately 34% in blasted florets between the most resistant and the most susceptible varieties. The magnitude of these differences suggests that they are real rather than apparent.

Two varieties of *A. byzantina*, Fulghum and Awnless Rustproof, appear to be the most resistant, while Ferguson Navarre, a variety of *A. sativa*, appears to be one of the most susceptible. White Cross and Gopher, two commonly grown varieties of *A. sativa*, also appear to be quite susceptible.

The percentage of blast on 97 oat varieties was correlated with the mean percentage of halo-blight on the same varieties. A non-significant coefficient of correlation of +.190 was obtained. If the values for blast and halo-blight infection used in obtaining this correlation truly represent the reactions of the varieties concerned, it would appear from the correlation value obtained that different sets of genetic factors are concerned in resistance to these two diseases.

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STUDIES ON THE CONTROL OF ROOT-ROT DISEASES OF CEREALS

IV. INFLUENCE OF MECHANICAL SEED INJURY ON INFECTION BY *FUSARIUM CULMORUM* IN WHEAT[1]

By J. E. Machacek[2] and F. J. Greaney[3]

Abstract

The results of field experiments made in 1932, 1933, and 1934, to determine the effect of mechanical seed injury on the incidence of root rot caused by *Fusarium culmorum* and on yield in wheat are presented.

Successful positive attacks of Fusarium root rot were experimentally induced in field plots. The tests showed that reduced emergence, increased root rot, and reduced yield uniformly followed the planting of injured wheat seed; and that the amount of disease increased and the yield decreased with an increase in the degree of seed injury. In these experiments Mindum and Marquis wheat seemed equally affected by seed injury.

The investigation suggests that the large annual losses in yield caused by root-rot diseases of cereals in Western Canada may be substantially reduced by sowing clean, vigorous, sound seed.

Introduction

A short account (4) of greenhouse and field experiments which were designed to determine the effect of mechanical seed injury on the incidence of Fusarium root rot in cereals was published in 1933. The greenhouse tests showed that seedlings arising from mechanically injured seed of wheat, oats, and barley were more frequently and severely attacked by *Fusarium culmorum* (W.G.Sm.) Sacc. than were seedlings from uninjured seed. The field tests (one year's results) demonstrated that the use of mechanically injured seed promoted the development of seedling blight and root rot in wheat, and thereby resulted in a retardation of plant growth and a reduction in yield. Continuing the field work of 1932, experiments were made at Winnipeg, Man., in 1933 and 1934. A summary of the three years' results is presented in this paper. The relevant literature on the subject of seed injury in wheat and other cereals has been reviewed in earlier papers (4, 5).

Experimental Methods

During the investigation, the following kinds of seed of Mindum and Marquis wheat were used: (1) Normal, sound seed (uninjured), (2) Lightly scarified seed (slightly injured), (3) Heavily scarified seed (severely injured).

Each experiment consisted of rod-row plots of injured and uninjured seed of these two varieties of wheat. One-half of each plot was artificially infested with *F. culmorum*.

The principles of randomization and replication were used in the design of the experiments and rendered possible valid tests of significance and experi-

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mental error. The data were analyzed by the procedure described by Fisher (1) as the analysis of variance. Details concerning plot arrangement, soil-infestation methods, and methods of recording the amount of disease infection in cereal root-rot experiments have already been presented (2).

In 1932 the seed coats were injured by rubbing the kernels with sandpaper. In 1933 and 1934 a small scarifying machine was used to injure the seed. The hand-made machine consisted of a wooden frame on which was mounted a small wooden roller, fitted with a hand crank, and an adjustable canvas apron which was pressed closely against one side of the roller. The roller and the surface of the apron facing the roller were covered with sandpaper. The kernels of wheat were passed between the revolving roller and the stationary apron. It was possible to obtain any required degree of injury to the seed by tightening or slackening the apron, and by passing the seed through the machine a given number of times.

The fungus *Fusarium culmorum* (W.G.Sm.) Sacc., which was originally isolated in 1930 from a rotted crown of Marquis wheat, was used to infest field plots in this study. Previous tests both in greenhouse and field had shown that this fungus was distinctly pathogenic to wheat.

Experimental Results

The value of the analysis of variance as a means of sorting out the effects of seed treatments, soil treatments, and varieties, and of eliminating irrelevant data in the experiments is illustrated in Table I. This table gives the complete analyses of variance for disease rating and yield data of the 1934 experiment.

TABLE I

ANALYSES OF VARIANCE. INJURED SEED EXPERIMENT, 1934

Variance due to	Degrees of freedom	Sum of squares	Mean square	Z	5% point
Disease Rating					
Blocks (between)	5	1,684.98	336.99		
Varieties	1	432.70	432.70	1.0167	0.9441
Error (a)	5	283.23	56.64		
Soil treatments	1	5,747.70	5,757.70	2.2141	0.8012
Soil treatments × varieties	1	52.54	52.54		
Error (b)	10	686.09	68.60		
Seed treatments	2	1,822.38	911.19	2.1210	0.7037
Seed treatments × varieties	2	4.08	2.04		
Seed treatments × soil treatments	2	40.58	20.29		
Seed treatments × varieties × soil treatments	2	23.05	11.52		
Error (c)	40	523.48	13.09		
Total	71	11,300.81			

TABLE I—*Concluded*

ANALYSES OF VARIANCE. INJURED SEED EXPERIMENT, 1934—*Concluded*

Variance due to	Degrees of freedom	Sum of squares	Mean square	Z	5% point
Yield					
Blocks (between)	5	1,237.08	247.42		
Varieties	1	4,371.12	4,371.12	2.5566	0.9441
Error (a)	5	131.29	26.25		
Soil treatments	1	3,954.56	3,954.56	2.4357	0.8012
Soil treatments × varieties	1	201.34	201.34		
Error (b)	10	303.38	30.33		
Seed treatments	2	1,272.07	636.03	1.7310	0.7037
Seed treatments × varieties	2	329.51	164.75		
Seed treatments × soil treatments	2	37.99	18.99		
Seed treatments × varieties × soil treatments	2	81.21	40.60		
Error (c)	40	797.87	19.95		
Total	71	12,717.42			

The analyses in Table I clearly establish significant differences, both with respect to disease rating and yield, between soil treatments, varieties, and seed treatments. In every instance the Z values are much greater than the 5% probability values. The results indicate, therefore, that a high degree of significance can be attached to differences in the amount of disease and yield observed in this experiment. A similar detailed examination was made of the data for 1932 and 1933. Standard errors for disease rating and yield for each of the three years are presented in the summary tables (Tables II, III, and IV).

Soil Treatments

It is known that the presence of a large and active natural fungus flora in soil has an appreciable influence on the development of parasitic fungi which attack the basal parts of cereal plants (3, 6, and others). On this account it is extremely difficult to determine to what extent the actual injury to a crop is caused by a particular organism introduced into a soil. To provide an adequate test of the effects of seed injury on the development of root rot caused by *F. culmorum*, the soil in one-half of the plots was experimentally infested with this organism. The experiments were designed so that differences in the amount of disease and in yield, arising from this type of soil treatment, could be properly evaluated. The importance of plot arrangement in cereal root-rot experiments has already been demonstrated elsewhere (2).

The effect on root-rot infection and on yield, of planting injured and uninjured seed of wheat in soil that was experimentally infested with *F. culmorum*, as well as in soil which was not, is shown in Table II.

TABLE II

EFFECT OF INTRODUCING *Fusarium culmorum* INTO THE SOIL OF FIELD PLOTS ON THE INCIDENCE OF ROOT ROT AND ON YIELD IN WHEAT IN 1932, 1933 AND 1934

Year	Soil treatment	Degree of seed injury			Mean	Z	5% point	Standard error of means
		Uninjured (control)	Slightly injured	Severely injured				
Disease Rating								
1932	Infested	72.1	—	77.4	74.7	1.69	0.72	0.90
	Control	66.1	—	68.2	67.2			
1933	Infested	91.3	93.7	96.9	94.0	2.98	0.71	1.02
	Control	60.5	67.1	69.4	65.7			
1934	Infested	70.4	77.6	81.6	76.5	2.21	0.80	1.38
	Control	52.2	58.0	65.7	58.6			
Yield (Bushels per acre)								
1932	Infested	33.4	—	29.7	31.5	1.52	0.72	0.96
	Control	38.9	—	35.7	37.3			
1933	Infested	10.6	6.5	2.9	6.7	2.75	0.71	0.56
	Control	19.7	19.9	17.3	19.0			
1934	Infested	20.0	12.1	10.9	14.3	2.44	0.80	0.92
	Control	34.9	28.7	23.9	29.2			

Severe attacks of Fusarium root rot were successfully induced in field plots of wheat in 1932, 1933 and 1934. The effectiveness of the method of applying mycelium and spores of *F. culmorum* to the seed and soil to induce a positive attack of root rot was established with a very high degree of probability. Each year the amount of root-rot infection was appreciably and significantly increased by artificial infestation of the soil. In 1933 and 1934 the yield difference between experimentally infested plots and those not so infested amounted to more than 12 bushels per acre.

Injured and Uninjured Seed

The evidence relating to the effect of planting mechanically injured seed in soil experimentally infested with *F. culmorum* and in ordinary field soil on the development of root rot and on yield in wheat is presented in Table III. During the three years 1932–34, the amount of root-rot infection was appreciably increased and the yield considerably decreased by sowing injured seed of Mindum and Marquis wheat. In all experiments the detrimental effects of planting injured seed in soil infested with *F. culmorum* were established with a high degree of significance. The results of 1933 and

TABLE III

EFFECT OF PLANTING MECHANICALLY INJURED SEED IN SOIL ARTIFICIALLY INFESTED WITH *Fusarium culmorum* AND IN ORDINARY FIELD SOIL ON THE INCIDENCE OF ROOT ROT AND ON YIELD IN WHEAT

Degree of mechanical seed injury	1932		1933		1934	
	Infested soil	Control soil	Infested soil	Control soil	Infested soil	Control soil
Disease Rating						
Uninjured	72.1	66.1	91.3	60.5	70.4	52.2
Slight	—	—	93.7	67.1	77.6	58.0
Severe	77.4	68.4	96.9	69.4	81.6	65.7
Standard error*	1.01		1.68		1.04	
Yield (Bushels per acre)						
Uninjured	33.4	38.9	10.6	19.7	20.0	34.9
Slight	—	—	6.5	19.9	12.1	28.7
Severe	29.7	35.7	2.9	17.3	10.9	23.9
Standard error*	1.51		1.22		1.29	

* *To be significant the difference between two quantities should exceed* $2 \times \sqrt{2} \times$ *standard error.*

1934 show that the amount of disease increased and the yield decreased with a progressive increase in the degree of injury. In 1932, experimentally infested plots planted with injured seed yielded 3.7 bushels per acre less than did plots sown with uninjured seed. The employment of severely injured seed in 1933 resulted in a loss of 7.7 bushels per acre; while in 1934 the yield loss per acre reached 9.1 bushels.

Varieties

Data showing the response of injured and uninjured seed of Mindum and Marquis wheat to root rot caused by *F. culmorum* are presented in Table IV. It is evident from this table that, in 1932 and 1933, Mindum was more susceptible to root rot than was Marquis. The significance of the difference observed in the amount of disease on these two varieties in 1934 is not clearly established. The results for 1932 and 1933 portray the most commonly occurring condition in Manitoba, for Mindum is usually more susceptible to Fusarium root rot than is Marquis. In both varieties and in all experiments the amount of disease was increased and the yield decreased by increasing the degree of injury. There is no conclusive evidence, however, that, as a result of mechanical injury to the seed, one variety suffered more than the other from root rot.

TABLE IV

THE EFFECT OF SEED INJURY ON MINDUM AND MARQUIS WHEAT IN RELATION TO THE INCIDENCE OF FUSARIUM ROOT ROT AND YIELD

Year	Variety	Degree of seed injury			Mean	.Z	5% point	Standard error of means
		Uninjured (control)	Slightly injured	Severely injured				
Disease Rating								
1932	Mindum	72.4	—	76.4	74.4	1.68	0.86	1.26
	Marquis	65.8	—	69.3	67.6			
1933	Mindum	78.3	80.8	85.4	81.5	0.89	0.73	0.97
	Marquis	73.5	80.0	80.9	78.1			
1934	Mindum	58.6	65.7	71.1	65.1	1.02	0.94	1.25
	Marquis	64.0	67.8	73.6	68.5			
Yield (Bushels per acre)								
1932	Mindum	43.4	—	40.5	41.9	2.05	0.86	1.91
	Marquis	28.9	—	25.0	27.0			
1933	Mindum	13.6	11.5	7.8	11.0	1.29	0.73	0.70
	Marquis	16.7	14.9	12.2	14.6			
1934	Mindum	38.2	27.1	23.3	29.5	2.55	0.94	0.84
	Marquis	16.7	13.6	11.6	14.0			

Discussion

In relation to the subsequent crop, the main effect of seed injury, such as that caused by frost, drought, rust, and drastic chemical treatment is that the seedlings are less vigorous than are those from sound seed. The same is true of seed mechanically injured by threshing or scarification, but with the former types of injury the seed coat usually remains intact, and constitutes a barrier to fungus invasion; whereas in the case of mechanical injury the seed coat is ruptured, the endosperm is exposed, an easy avenue for fungus invasion is provided. This invasion may occur if the seed is stored under unfavorable conditions. In Western Canada, however, where storage conditions do not favor moldiness of grain, the invasion of the endosperm of injured seed by saprophytic and parasitic fungi is most likely to take place after the seed has been planted in the soil.

There is in the prairie soils of Western Canada a large and active fungus flora. Parasitic forms, particularly root-rotting fungi, are commonly present in all the major wheat-producing soils. These fungi can be expected to cause serious damage whenever environmental conditions favor their attack. As mechanical injury to the seed of wheat, and to the seed of other cereal crops, renders the endospermic nutrients accessible to saprophytic and parasitic

soil fungi, growth of these organisms is thus favored at the expense of the seedling plant with the result that plant emergence is reduced and a high percentage of the weak seedlings that do emerge fall an easy prey to the parasitic organisms. The final result is an appreciable reduction in yield.

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HEAT INDUCED TETRAPLOIDY IN BARLEY[1]

By F. H. Peto[2]

Abstract

A tetraploid sector on a barley spike was induced by germinating and growing barley for seven days at 35° C. Tetraploid and diploid florets from the same spike were compared with respect to chromosome pairing and chiasma formation.

Brief reference has already been made to a barley plant with a tetraploid sector produced in the summer of 1934 (1). In view of the interest shown by cytologists, it seems desirable to publish further details.

O.A.C. 21 barley was germinated and grown at 35° C. for seven days and subsequently carried to maturity under normal growth conditions. The treatment reduced germination to 62%, and 46% of the seedlings died, while the seedling growth rate of the remainder was severely checked.

The meiotic behavior of twenty of the above plants was studied and all but one found normal. In this plant both tetraploid and diploid florets were observed and their positions indicated that one side of the spike was tetraploid and the other side normal diploid. Chromosome doubling must therefore have occurred in a somatic division immediately subsequent to the differentiation of the primordium of the spike.

The occurrence of both tetraploid and diploid florets in the same spike afforded a unique opportunity for comparing these with respect to chromosome pairing and chiasma formation, since both types of florets had been exposed to identical internal as well as external environmental conditions and possessed identical genetical constitutions. Twelve nuclei of each of the two types of florets were analyzed and the data are given in Table I. The number of quadrivalents per nucleus varied from 1 to 7 with a mean of 3.58 while the number of bivalents varied from 2 to 12 with a mean of 6.75 On the average, approximately one-half of the chromosomes paired as quadrivalents and the other half as bivalents, with occasional univalents. Sixty-six cells were examined between mid-anaphase and early telophase in the heterotypic division; eleven of these had one pair of lagging chromosomes and two had three pairs of lagging chromosomes. Presumably most of these lagging chromosomes were the result of failure to pair, and a somewhat greater number of univalents might have been observed at metaphase had it been possible to analyze a larger number of nuclei.

The pairing in the diploid florets was very regular; seven bivalents were always present and all other meiotic stages were normal.

The number of chiasmata in the tetraploid nuclei should be double that observed in the diploid nuclei. Actually, 1.87 times as many chiasmata were present. Thus it appears that the presence of four instead of two

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TABLE I

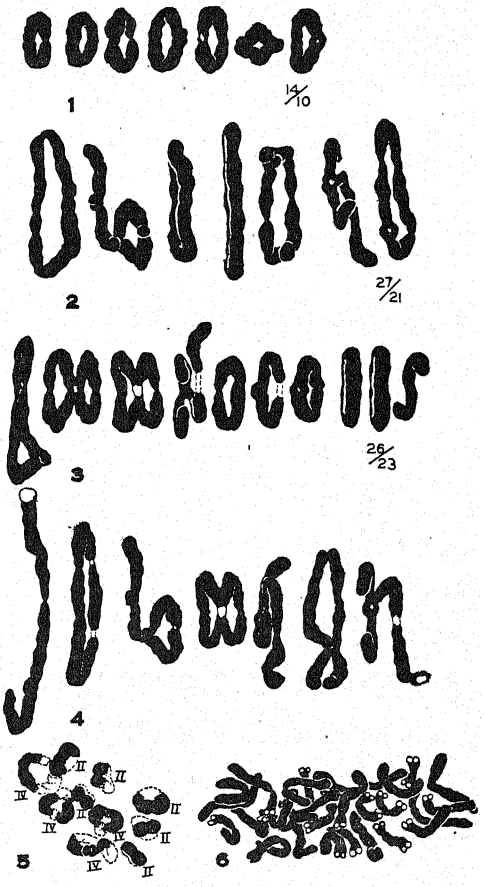
ANALYSIS OF HETEROTYPIC METAPHASE CONFIGURATIONS

No.	Univalents	Bivalents	Quadrivalents	Chasmata	
				Total	Terminal
			Tetraploid nuclei		
1	0	10	2	23	19
2	0	12	1	26	22
3	2	7	3	26	24
4	0	6	4	23	21
5	0	6	4	27	24
6	0	8	3	27	26
7	0	0	7	27	21
8	0	6	4	27	23
9	0	2	6	26	19
10	0	10	2	27	21
11	0	6	4	26	23
12	0	8	3	28	23
Total	2	81	43	313	266
Mean	0.17	6.75	3.58	26.08	22.17
			Diploid nuclei		
1		7		14	12
2		7		14	13
3		7		14	9
4		7		14	10
5		7		13	9
6		7		14	12
7		7		14	11
8		7		14	12
9		7		14	12
10		7		14	12
11		7		14	10
12		7		14	13
Total		84		167	135
Mean		7		13.92	10.41

homologous chromosomes caused only a very limited amount of interference in zygotene pairing. The random attraction of homologous regions at zygotene would account for the proportions of quadrivalents and bivalents observed. However, it is important to note that trivalents and single univalents never appeared. This would be expected on the assumption that prophase chromosomes are only attracted in pairs in any given homologous region. Consequently the pairing attraction throughout the whole chromosome length cannot be satisfied in trivalent formation. In any three chromosomes that tended to pair together at zygotene, there would always be at least half the length of one or two of them in which the attraction would be unsatisfied and these portions consequently would be free to pair with the fourth homologue.

Examples of diploid and tetraploid p.m.c. nuclei are shown in Figs. 1 to 3. Seven different types of quadrivalent associations are shown in Fig. 4 and represent almost all of the known types. Two tetraploid megaspore-mother cells were also analyzed; one contained seven quadrivalents and the other contained four quadrivalents and six bivalents (Fig. 5). The somatic tissues of the florets were also examined and chromosome counts taken wherever possible. A tetraploid nucleus from the nucellus is shown in Fig. 6.

It was unfortunate that this spike was completely used for cytological examination, otherwise there seems little doubt that a tetraploid strain of barley could have been established. However, the fact that a heat treatment during germination can induce somatic chromosome doubling in the primordium of a spike is of considerable importance. At the present time there is need for a satisfactory technique for conferring fertility on sterile F_1 hybrids by doubling the chromosome number in the primordia of spikes. This study gives some hope that such a technique can be developed.



FIGS. 1–6. FIG. 1. *Diploid pollen-mother cell, 7 bivalents.* FIG. 2. *Tetraploid p.m.c., 7 quadrivalents.* FIG. 3. *Tetraploid p.m.c., 4 quadrivalents and 6 bivalents.* FIG. 4. *Various types of quadrivalents.* FIG. 5. *Megaspore-mother cell, 4 quadrivalents and 6 bivalents.* FIG. 6. *Tetraploid nucleus from the nucellus. Magnifications* × *2000.*

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